



(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
26.01.2022 Bulletin 2022/04

(21) Application number: **21193195.1**

(22) Date of filing: **24.02.2015**

(51) International Patent Classification (IPC):

A61K 35/35 ^(2015.01)	A61K 38/18 ^(2006.01)
A61K 38/19 ^(2006.01)	A61K 38/20 ^(2006.01)
A61K 38/22 ^(2006.01)	A61K 31/05 ^(2006.01)
A61K 31/165 ^(2006.01)	A61K 31/166 ^(2006.01)
A61K 31/195 ^(2006.01)	A61K 31/352 ^(2006.01)
A61K 31/426 ^(2006.01)	A61K 31/4375 ^(2006.01)
A61K 31/4422 ^(2006.01)	A61K 31/475 ^(2006.01)
A61K 31/4965 ^(2006.01)	A61K 31/506 ^(2006.01)
A61K 31/5415 ^(2006.01)	A61K 31/5575 ^(2006.01)
A61K 31/603 ^(2006.01)	C12N 5/077 ^(2010.01)

(52) Cooperative Patent Classification (CPC):

**A61K 35/35; A61K 31/05; A61K 31/165;
A61K 31/166; A61K 31/195; A61K 31/352;
A61K 31/426; A61K 31/4375; A61K 31/4422;
A61K 31/475; A61K 31/4965; A61K 31/506;
A61K 31/5415; A61K 31/5575; A61K 31/603;**

(Cont.)

(84) Designated Contracting States:
**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO RS SE SI SK SM TR**

(30) Priority: **24.02.2014 US 201461966496 P**

(62) Document number(s) of the earlier application(s) in
accordance with Art. 76 EPC:
15752698.9 / 3 110 946

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Remarks:

This application was filed on 26-08-2021 as a
divisional application to the application mentioned
under INID code 62.

(54) **COMPOSITIONS FOR USE IN INDUCING DIFFERENTIATION OF HUMAN BROWN ADIPOCYTE
PROGENITORS**

(57) This disclosure relates to compositions and
methods for recruiting brown adipocytes in vitro from
brown adipocyte progenitor cells found in human skeletal
muscle. Said compositions for use in methods for treating
metabolic disease are also provided. In some embodi-

ments, the brown adipocyte recruiter is a human protein
or peptide. In other embodiments the brown adipocyte
recruiter may be a non-human protein or peptide. In still
other embodiments, the brown adipocyte recruiter is a
small molecule or natural product.

(52) Cooperative Patent Classification (CPC): (Cont.)

**A61K 38/1825; A61K 38/195; A61K 38/204;
A61K 38/2242; A61P 1/16; A61P 3/00;
A61P 3/04; A61P 3/06; A61P 3/10; A61P 5/00;
A61P 5/50; A61P 9/12; A61P 15/00; A61P 15/08;
A61P 17/00; A61P 19/06; A61P 25/00;
A61P 25/28; A61P 43/00; C12N 5/0653;
C12N 2503/02**

Description**CROSS REFERENCE TO RELATED APPLICATIONS**

- 5 **[0001]** This claims priority to and the benefit of U.S. Provisional Application No. 61/966,496 filed February 24, 2014, the entire disclosure of which is incorporated herein by reference.

TECHNICAL FIELD

- 10 **[0002]** The present disclosure relates to compositions and methods related to enhancing brown adipocytes, and/or brown adipocyte mass, in conditions such as type 2 diabetes, obesity, insulin-resistance, and dyslipidemia. Specifically, the present disclosure identifies and describes compounds that increase or promote the differentiation of brown adipose tissue (BAT) progenitor cells isolated from skeletal muscle into brown adipocytes. Further, the present disclosure identifies and describes compounds which interact with gene products involved in the regulation of brown adipocyte differentiation and/or mass. Still further, the present disclosure provides methods for the identification and therapeutic use of compounds for the prevention and treatment of type 2 diabetes, obesity, insulin-resistance, and dyslipidemia. The disclosure is useful for the study, prevention, and treatment of various metabolic diseases such as obesity, type 2 diabetes, insulin-resistance and dyslipidemia.

20 **BACKGROUND**

- [0003]** The epidemic of obesity is closely associated with increases in the prevalence of diabetes, hypertension, coronary heart disease, cancer and other disorders. The role of white adipose tissue is to store lipids, and it is associated with obesity. The role of brown adipose tissue ("BAT") is effectively the opposite. It is specialized in lipid combustion and the dissipation of energy as heat. Indeed, the brown adipocyte contains numerous mitochondria (in which cellular combustion occurs) and uniquely expresses uncoupling protein-1 ("UCP1"). UCP1 acts as an uncoupler of oxidative phosphorylation, resulting in dissipation of energy as heat. The sympathetic nervous system stimulates mitochondriogenesis and UCP1 expression and activity. BAT-associated thermogenesis in rodents is increased upon exposure to low temperature (e.g., preventing hypothermia) or as a result of overeating, burning excess absorbed fat and preventing weight gain. BAT, by modifying susceptibility to weight gain and by consuming large amounts of glucose, also improves insulin sensitivity. It therefore plays an important role in the maintenance of body temperature, energy balance and glucose metabolism.

- [0004]** Experiments with transgenic animals support the potential anti-obesity properties of BAT. For example, the genetic ablation of BAT has been reported to cause obesity, while genetic increase in the amount and/or function of BAT (and/or UCP1 expression) reportedly promotes a lean and healthy phenotype. Specifically, mice with a higher amount of BAT gain less weight and are more insulin-sensitive than control mice. Recently, ectopic BAT depots were evidenced in the mouse muscle, which have been shown to provide a genetic mechanism of protection from weight gain and metabolic syndrome.

- 40 **[0005]** Although UCP1 is reported to play a role in the control of energy balance in rodents and UCP1-expressing BAT is present in human neonates, it has long been thought that there was no physiologically relevant UCP1 expression in adult humans. Indeed, UCP1-expressing BAT was thought to disappear early in life, and adult humans were thought to be devoid of BAT. Recently however, numerous studies have demonstrated that BAT is indeed maintained in most adult humans, albeit at considerably lower levels than in neonates and children.

- 45 **[0006]** As such, a need exists to carefully identify and study ways to provide more BAT in the adult body and/or stimulate UCP1 expression, for the study, prevention and treatment of various metabolic diseases such as obesity, type 2 diabetes, insulin-resistance, dyslipidemia and type 1 diabetes.

- [0007]** Applicants previously identified the presence of cells in various tissues that are capable of differentiating into brown adipocytes in e.g., PCT Publication No. WO2009151541 and WO2013071063, the entire disclosures of both of which are incorporated herein by reference. However, a need exists for agents (e.g., compounds, proteins, biologicals, and the like) that can, for example, induce the expression of the UCP1 gene, promote the differentiation of BAT progenitor cells into brown adipocytes in vitro, promote the differentiation of BAT progenitor cells to brown adipocytes in vivo, or combinations of these activities.

SUMMARY

- 55 **[0008]** The present disclosure provides compositions for recruiting or producing brown adipocytes in vitro and in vivo from BAT progenitor cells found in human skeletal muscle. These agents, or combinations thereof, can be used to promote the differentiation of BAT progenitor cells into brown adipocytes and/or induce the expression of UCP1, FABP4

(aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in BAT progenitor cells in vitro, in vivo, or both. Furthermore, these agents can be used to treat metabolic disease, including without limitation obesity, diabetes, insulin resistance, hyperlipidemia, and others conditions in a patient.

[0009] The present disclosure is based, in part, on the discovery that various proteins, peptides, and small molecules (collectively, agents) play an important role in the differentiation of BAT progenitor cells. In particular, it has been found that the various agents disclosed herein markedly induce differentiation of BAT progenitor cells isolated from human skeletal muscle into mature, functional brown adipocytes. Treatment of these BAT progenitor cells with one or more of these various agents triggers commitment of these cells to brown adipocyte differentiation. In some cases, treatment with an agent for a period of time (e.g., a few hours, 1 day, 2 days, 3 days, or shorter or longer) prior to the introduction of an adipogenic medium results in brown adipocyte differentiation. In other cases, treatment with an agent contemporaneously with, and/or after the introduction of adipogenic medium results in brown adipocyte differentiation.

[0010] Since brown adipose tissue (BAT) is specialized for energy expenditure, the agents described herein are useful for the treatment of obesity and related disorders, such as diabetes. The agents can also be used to decrease fat stores in subjects including food animals, e.g., to improve the quality of the meat derived therefrom.

[0011] Accordingly, in one aspect, the disclosure features a method of treating a subject, e.g., decreasing fat stores or weight in a subject such as a human. The method includes administering to the subject an effective amount of an agent or combination of agents disclosed herein.

[0012] In a further aspect, the disclosure features a method of treating a subject in need of decreasing fat stores or weight, by administering a population of agent-activated BAT progenitor cells, wherein said population of agent-activated progenitor cells undergo brown adipogenesis. The method can optionally include identifying a subject in need of decreasing fat stores or weight.

[0013] In a further aspect, the disclosure includes a method of enhancing insulin sensitivity in a subject, e.g., a subject that is insulin-resistant. The method includes administering to the subject an agent and/or a population of agent-activated BAT progenitor cells, wherein said population of agent-activated BAT progenitor cells undergo brown adipogenesis. The method can optionally include identifying a subject in need of enhanced insulin sensitivity.

[0014] In another aspect, the disclosure features a method of modulating brown adipose tissue function or development, e.g., promoting BAT adipogenesis, in a subject. The method includes administering to the subject an agent and/or a population of agent-activated BAT progenitor cells, wherein said population of agent-activated progenitor cells undergo brown adipogenesis.

[0015] In some embodiments, methods described herein can include implanting a population of agent-activated BAT progenitor cells into a subject. The agent-activated cells can be implanted directly or can be administered in a scaffold, matrix, or other implantable device to which the cells can attach (examples include carriers made of, e.g., collagen, fibronectin, elastin, cellulose acetate, cellulose nitrate, polysaccharide, fibrin, gelatin, self-assembling small peptides, and combinations thereof). In general, the methods include implanting a population of agent-activated BAT progenitor cells comprising a sufficient number of cells to promote increased brown adipocyte mass in the subject, e.g., to increase the amount of brown adipocytes in the subject by at least 1%, e.g., 2%, 5%, 7%, 10%, 15%, 20%, 25% or more.

[0016] In some embodiments, the methods include evaluating the level of BAT adipogenesis in a subject, by contacting isolated BAT progenitor cells from a subject with one or more of the agents disclosed herein. BAT differentiation can be evaluated by measuring any of, e.g., a BAT marker, such as uncoupling protein (UCP), e.g., UCP1, expression; BAT morphology (e.g., using visual, e.g., microscopic, inspection of the cells); or BAT thermodynamics, e.g., cytochrome oxidase activity, Na⁺-K⁺-ATPase enzyme units, or other enzymes involved in BAT thermogenesis.

[0017] In general, the subject can be a mammal. In some embodiments, the subject is a human subject, e.g., an obese human subject. In some embodiments, the subject is a non-human mammal, e.g., an experimental animal, a companion animal, or livestock, e.g., a cow, pig, or sheep that is raised for food. Generally, where a protein or peptide is used to recruit brown adipocytes from BAT progenitor cells, the protein or peptide will be from the same or related species as the subject, e.g., human, cat, dog, cow, pig, or sheep. The protein or peptide can also be heterologous to the subject.

[0018] In some embodiments, the methods include evaluating the subject for one or more of: weight, white adipose tissue stores, brown adipose tissue stores, adipose tissue morphology, insulin levels, insulin metabolism, glucose levels, thermogenic capacity, and cold sensitivity. The evaluation can be performed before, during, and/or after the administration of the agent and/or agent-activated BAT progenitor cells. For example, the evaluation can be performed at least 1 day, 2 days, 4 days, 7 days, 14 days, 21 days, 30 days or more or less before and/or after the administration.

[0019] In some embodiments, the methods include one or more additional rounds of treatment with an agent or implantation of agent-activated BAT progenitor cells, e.g., to increase brown adipocyte mass, e.g., to maintain or further reduce obesity in the subject.

[0020] In some embodiments where a protein or peptide agent is used, BAT progenitor cells can be genetically engineered to express increased levels of such protein or peptide, either stably or transiently. The cells can be, e.g., cultured mammalian cells, e.g., human cells. The expressed recombinant protein or peptide used will generally be of the same or related species as the BAT progenitor cells, e.g., a human protein or peptide expressed in human cells. The recombinant

protein or peptide can also be heterologous to the BAT progenitor cells.

[0021] In a further aspect, the present disclosure provides use of one or more agents disclosed herein, for promoting differentiation of BAT progenitor cells into brown adipocytes and/or inducing expression of UCP1, FABP4 (aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in BAT progenitor cells in vitro, in vivo, or both.

[0022] Also provided herein is use of one or more agents disclosed herein, for the treatment of one or more diseases or conditions selected from overweight, obesity, insulin resistance, diabetes, hyperinsulinemia, hypertension, hyperlipidemia, hepatosteatosis, fatty liver, non-alcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome, acanthosis nigricans, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Laurence-Moon syndrome, Prader-Willi syndrome, neurodegenerative diseases, and Alzheimer's disease. Methods for treating the foregoing diseases are also provided, comprising administering one or more agents disclosed herein to a subject in need thereof.

[0023] A further aspect relates to a pharmaceutical composition, comprising one or more agents disclosed herein, and a pharmaceutically acceptable excipient, diluent or carrier.

[0024] One particular aspect relates to use of an agent for recruiting brown adipocytes from BAT progenitor cells isolated from human skeletal muscle, wherein the agent is selected from one or more of:

an antihistamine such as Famotidine;
 an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine or Spiperone;
 a ligand of tubulin such as Colchicine;
 a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
 a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP Propeptide of 55 aa (aa 25-79);
 a flavonoid such as kaempferol;
 a fibroblast growth factor (FGF) such as FGF7 or FGF10 or FGF13;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 bombesin;
 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ;
 a cyclooxygenase inhibitor such as Diflunisal;
 a biguanide such as Metformin;
 a phosphodiesterase inhibitor such as a PDE3 inhibitor such as siguazodan;
 a stimulator of soluble guanylate cyclase (sGC) such as riociguat;
 b-type natriuretic peptide (BNP);
 ciliary neurotrophic factor (CNTF);
 interleukin-6 (IL-6);
 orexin B; and
 an α 2 adrenergic receptor agonist such as Guanfacine hydrochloride.

[0025] In some embodiments, the agent is selected from one or more of:

an antihistamine such as Famotidine;
 an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine;
 a ligand of tubulin such as Colchicine;
 a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
 a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP Propeptide of 55 aa (aa 25-79);
 a flavonoid such as kaempferol;
 a fibroblast growth factor (FGF) such as FGF7 or FGF10;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 bombesin;
 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ; and

a cyclooxygenase inhibitor such as Diflunisal.

[0026] In certain embodiments, the agent is capable of inducing expression of UCP1, FABP4 (aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in the BAT progenitor cells in vitro, in vivo, or both.

[0027] In some embodiments, the agent can have one or more biological activities selected from the group consisting of:

(a) causing an increase or decrease in one or more of the following: Beta-3 adrenergic receptor (β 3-AR), Solute carrier family 2, facilitated glucose transporter member 4 (SLC2A4), Elongation of very long chain fatty acids protein 3 (ELOVL3), CD36 antigen, Type II iodothyronine deiodinase (DIO2), BMP5, BMP6, FGF7, FGF10, FGF13, FGF21, Fatty acid binding protein 7 (FABP7), CXCL12, Atypical chemokine receptor 3 (ACKR3), Insulin-like growth factor-binding protein 4 (IGFBP4), Pituitary adenylate cyclase-activating polypeptide (PACAP), Adenylate cyclase 4 (ADCY4), Cell death activator CIDE-A (CIDEA), secreted frizzled-related protein 1 (SRFP1), SRFP2, brain-derived neurotrophic factor (BDNF), Vascular endothelial growth factor D (VEGF-D), Transforming growth factor beta-2 (TGFB2), cAMP-specific 3',5'-cyclic phosphodiesterase 4B (PDE4B), cAMP-specific 3',5'-cyclic phosphodiesterase 4D (PDE4D), High affinity cAMP-specific 3',5'-cyclic phosphodiesterase 7A (PDE7A), and cAMP-specific 3',5'-cyclic phosphodiesterase 7B (PDE7B);

(b) causing an increase in thermogenesis in brown adipose tissue and/or skeletal muscle tissue;

(c) causing an increase in insulin sensitivity of skeletal muscle, white adipose tissue, or liver;

(d) causing an increase in glucose tolerance;

(e) causing an increase in basal respiration, maximal respiration rate, or uncoupled respiration;

(f) causing an increase in metabolic rate; and

(g) causing a decrease in hepatosteatosis.

[0028] In certain embodiments, the agent can cause an increase or decrease in one or more of the following: Beta-3 adrenergic receptor (β 3-AR), Solute carrier family 2, facilitated glucose transporter member 4 (SLC2A4), Elongation of very long chain fatty acids protein 3 (ELOVL3), CD36 antigen, and Type II iodothyronine deiodinase (DIO2).

[0029] In some embodiments, the agent is capable of modulating a metabolic response in a subject or preventing or treating a metabolic disorder in a subject. The metabolic disorder can, in some embodiments, be one or more of obesity, type II diabetes, insulin resistance, hyperinsulinemia, hypertension, hyperlipidemia, hepatosteatosis, fatty liver, non-alcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome, acanthosis nigricans, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Laurence-Moon syndrome, Prader-Willi syndrome, neurodegenerative diseases, and Alzheimer's disease.

[0030] Another aspect relates to a method of promoting brown adipogenesis in a subject in need thereof, the method comprising administering to the subject an agent selected from one or more of agents disclosed herein. The method in some embodiments can further include modulating a metabolic response in the subject and/or preventing or treating a metabolic disorder in the subject. The metabolic disorder may be one or more of obesity, type II diabetes, insulin resistance, hyperinsulinemia, hypertension, hyperlipidemia, hepatosteatosis, fatty liver, non-alcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome, acanthosis nigricans, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Laurence-Moon syndrome, Prader-Willi syndrome, neurodegenerative diseases, and Alzheimer's disease. In certain embodiments, the method further includes contacting a cell of the subject with the agent, and optionally transplantation of said cell into the subject after said contacting step. The cell in some embodiments can be a BAT progenitor cell isolated from human skeletal muscle. The cell can be positive for CD34 and/or negative for CD31.

[0031] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032]

FIG. 1 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of PPAR γ 2 mRNA.

FIG. 2 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of PPAR γ 2 mRNA.

FIG. 3 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of UCP1 mRNA.

FIG. 4 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of UCP1 mRNA.

FIG. 5 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0) on the expression of PPAR γ 2 mRNA.

FIG. 6 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0) on the expression of PPAR γ 2 mRNA.

FIG. 7 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0) on the expression of PPAR γ 2 mRNA.

FIG. 8 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of PPAR γ 2 mRNA.

FIG. 9 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of UCP1 mRNA.

FIG. 10 shows effects of FGF7 and FGF 10 (both 100 nM, incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of UCP1 mRNA.

FIG. 11 shows effects of FGF7 and FGF 10 (both 100 nM, incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of PPAR γ 2 mRNA.

FIG. 12 shows effects of FGF7 (1 nM, incubated with brown adipocyte progenitor cells at day 0 to d3) on the expression of UCP1 mRNA. Rosiglitazone (rosi, 1 μ M), bone morphogenic protein-7 (bmp7, 6 nM) or both rosi and bmp7 were incubated with the cells at day -3 to d0.

FIG. 13 shows effects of FGF7 (1 nM, incubated with brown adipocyte progenitor cells at day 0 to d3) on the expression of PPAR γ 2 mRNA. Rosiglitazone (rosi, 1 μ M), bone morphogenic protein-7 (bmp7, 6 nM) or both rosi and bmp7 were incubated with the cells at day -3 to d0.

FIG. 14 shows effects of FGF10 (10 nM, incubated with brown adipocyte progenitor cells at day -3 to d0) on the expression of UCP1 mRNA. Rosiglitazone (rosi, 1 μ M), bone morphogenic protein-7 (bmp7, 6 nM) or both rosi and bmp7 were incubated with the cells at day -3 to d0.

FIG. 15 shows effects of FGF10 (10 nM, incubated with brown adipocyte progenitor cells at day -3 to d0) on the expression of PPAR γ 2 mRNA. Rosiglitazone (rosi, 1 μ M), bone morphogenic protein-7 (bmp7, 6 nM) or both rosi and bmp7 were incubated with the cells at day -3 to d0.

FIG. 16 shows effects of FGF7 (1 nM, incubated with brown adipocyte progenitor cells at day 0 to d3) or FGF10 (10 nM, incubated with the cells at day -3 to d0) on the expression of UCP1 mRNA. Rosiglitazone (rosi, 1 μ M), bone morphogenic protein-7 (bmp7, 6 nM) or both rosi and bmp7 were incubated with the cells at day -3 to d0.

FIG. 17 shows effects of FGF7 (1 nM, incubated with brown adipocyte progenitor cells at day 0 to d3) or FGF10 (10 nM, incubated with the cells at day -3 to d0) on the expression of PPAR γ 2 mRNA. Rosiglitazone (rosi, 1 μ M), bone morphogenic protein-7 (bmp7, 6 nM) or both rosi and bmp7 were incubated with the cells at day -3 to d0.

FIG. 18 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of PPAR γ 2 mRNA.

FIG. 19 shows effects of various agents (incubated with brown adipocyte progenitor cells at day -3 to d0 and d0 to d3) on the expression of UCP1 mRNA.

FIGS. 20A-20C show fluorescence microscopy pictures illustrating the immunohistochemistry (IHC) assay results for UCP1 protein expression (FITC, green) and cell nuclei numbers (DAPI, blue). CD31- cells differentiated for 8 days in minimal differentiation medium (MDM) after exposure to rosiglitazone (1 μ M) differentiate profoundly into brown adipocytes expressing high levels of UCP1 (FIG. 20A), whereas cells not exposed to rosiglitazone show a much lower level of differentiation and UCP1 expression (FIG. 20B). Cells maintained in proliferation medium (EGM-2) do not differentiate and express no UCP1 (FIG. 20C).

FIG. 21 shows BODIPY 500/510 C1, C12 fluorescence signal after brown adipocyte progenitor cells were induced to differentiate in culture for 9 days in various conditions (rosi and BMP-7 were used from day -3 to d0).

FIG. 22 shows fluorescence microscopy illustrating the BODIPY assay results for intracellular lipid droplet formation (BODIPY 500/510 C1, C12, green). CD31- cells were differentiated for 8 days in minimal differentiation medium (MDM) after exposure to rosiglitazone (1 μ M) for 3 days (day -3 to day 0).

FIGS. 23A-23H show light microscopic photos of the CD31- cells and resulting brown adipocyte differentiation following treatment with several agents that promote brown adipocyte formation. FIG. 23A: Vehicle (DMSO). FIG. 23B: Rosiglitazone. FIG. 23C: BMP7. FIG. 23D: Diflunisal. FIG. 23E: Syrosingopine. FIG. 23F: Kaempferol. FIG. 23G: Probenecid. FIG. 23H: Tiapride.

DETAILED DESCRIPTION

[0033] As used herein, "agent-activated" means that the BAT progenitor cell or cells have been treated with one or more agents as described herein and are at least partially committed to differentiate into brown adipocytes. The cells can be autologous, allogeneic, or xenogeneic. "Brown adipogenesis" means generation of brown adipocytes from BAT progenitor cells in vivo, in vitro, or partially in vivo and partially in vitro. It should be noted that brown adipogenesis may be induced, i.e., the so-called "BAT progenitor cells" before induction by, e.g., one or more agents disclosed herein, was not necessarily committed to differentiate into brown adipocytes and may be reprogrammed or transdifferentiate into brown adipocytes from a stem cell or a somatic cell. "Recruiting brown adipocytes" means promoting or enhancing differentiation of BAT progenitor cells into brown adipocytes, and/or increasing the amount or concentration of brown adipocytes, in vivo and/or in vitro.

[0034] Provided herein are agents (e.g., compounds, proteins, biologicals, and the like) that can promote the differentiation of BAT progenitor cells into brown adipocytes and/or induce the expression of the UCP1 gene in vitro, in vivo, or both. Such agents can be identified by screening compounds, proteins, biologicals, and the like. For example, in some embodiments BAT progenitor cells (e.g., those isolated from human skeletal muscle) can be used to screen agents for the ability to induce expression of the UCP1 gene and/or differentiation of the BAT progenitor cells into brown adipocytes. Agents identified in this manner can be used for a variety of research, diagnostic and therapeutic purposes, including, for example, treatment of metabolic diseases such as obesity, type 2 diabetes, insulin-resistance, dyslipidemia, and the like. In some embodiments, an agent identified by an assay according to the present disclosure is optimized for improvement of its physico-chemical and/or pharmacokinetic properties.

[0035] Expression of UCP1, FABP4 (aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in BAT progenitor cells in vitro and in vivo can be enhanced according to methods provided in the present disclosure. In some embodiments, exposure to adipogenic media can be used to stimulate increased expression of UCP1, FABP4 (aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in BAT progenitor cells.

[0036] Accordingly, in some embodiments the following agents, or combinations thereof, can be used to promote the differentiation of BAT progenitor cells into brown adipocytes and/or induce the expression of UCP1, FABP4 (aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in BAT progenitor cells in vitro, in vivo, or both: a PDE3 inhibitor (e.g., siguazodan), a PDE4 inhibitor (e.g., rolipram), a derivative of prostaglandin F2 (PGF2) such as 9 β ,11 α -prostaglandin F2 or 9 α ,11 β -prostaglandin F2, a peptide derived (e.g., a portion) from the Pituitary adenylate cyclase-activating polypeptide (PACAP, ADCYAP1, UniProt P18509) gene such as the PACAP Propeptide of 55 aa (aa 25-79), BDNF (brain-derived neurotrophic factor), a TGR5 agonist such as oleanolic acid, BMP-7, a flavonoid such as kaempferol (KMP, CAS number 520-18-3), a stimulator of soluble guanylate cyclase (sGC) such as riociguat (BAY 63-2521, CAS 625115-55-1), fibroblast growth factors (such as FGF7 (fibroblast growth factor-7, KGF, keratinocyte growth factor), FGF 10 (fibroblast growth factor-10, KGF-2, keratinocyte growth factor-2), or FGF13 (fibroblast growth factor-13)), BNP (b-type natriuretic peptide), a TRPM8 (CMRI) ligand such as menthol or icilin, a bombesin peptide such as from the toad (UniProt P84214), CNTF (ciliary neurotrophic factor, UniProt P05231), interleukin-6 (IL-6), orexin B, SDF-1 γ (CXCL12), or Guanfacine hydrochloride.

[0037] Further, in other embodiments additional agents or combinations thereof that can be used to promote the differentiation of BAT progenitor cells into brown adipocytes and/or induce the expression of UCP1 include prostaglandin J2 (PGJ2), 24(S)-Hydroxycholesterol, forms of vitamin D such as 1,25-Dihydroxyvitamin D3 or 24,25-Dihydroxyvitamin D3, and a cyclooxygenase inhibitor such as Diflunisal.

[0038] In still other embodiments, additional agents or combinations thereof that can be used to promote the differentiation of BAT progenitor cells into brown adipocytes and/or induce the expression of UCP1 include bone morphogenetic proteins such as BMP5 (bone morphogenetic protein 5, UniProt P22003) and BMP6 (bone morphogenetic protein 6, UniProt P22004), Platelet-derived growth factor receptor-like protein (PDGFR, UniProt Q15198), Vascular endothelial growth factor D (VEGF-D, FIGF, UniProt 043915), CYTL1 (cytokine-like protein 1, UniProt Q9NRR1), SCG2 (secretogranin-2, UniProt P13521), NPTX2 (neuronal pentraxin-2, UniProt P47972), OLFML2B (olfactomedin-like protein 2B, UniProt Q68BL8), TFPI2 (tissue factor pathway inhibitor 2, UniProt P48307), IFNE (interferon epsilon, UniProt Q86WN2), a Prostaglandin F2- α receptor (PTGFR, prostanoid FP receptor, UniProt P43088) ligand such as prostaglandin F2, CNTF (ciliary neurotrophic factor), Interleukin-6 (IL-6, UniProt P05231), Interleukin-15 (IL-15, UniProt P40933), CXCL12 ((chemokine (C-X-C motif) ligand 12), stromal cell-derived factor 1, SDF1, UniProt P48061 isoform SDF-1g/UniProt P48061-3), and/or a ligand of Atypical chemokine receptor 3 (ACKR3, CMKOR1, CXCR7, GPR159, RDC1, UniProt P25106) such as SDF1 (CXCL12).

[0039] In still further embodiments, other agents or combinations thereof that can be used to promote the differentiation of BAT progenitor cells into brown adipocytes and/or induce the expression of UCP1 include a biguanide such as Metformin, an antihistamine such as Famotidine, an antidopaminergic such as Tiapride hydrochloride, Spiperone, Thioethylperazine, a microtubule modulator such as Colchicine, a Rauwolfia alkaloid or derivative of such as Reserpine or Syrosingopine, a potassium channel ligand such as Minoxidil, Probenecid, or a calcium channel antagonist such as

Felodipine.

[0040] In some embodiments, treatment of a subject, including a human subject, with one or a combination of agents shown here, results in an increase in the production of UCP1 mRNA or protein in the subject's skeletal muscle. For example, treatment of subjects with rosiglitazone can, in some embodiments, induce the appearance or differentiation of brown adipocytes in skeletal muscle, enhance expression of the UCP1 gene in existing brown adipocytes in or near skeletal muscle (between myofibers, at the surface of and/or adjacent to skeletal muscle tissue), or both. In some embodiments the appearance or differentiation of brown adipocytes in skeletal muscle can be induced in a subject suffering from a metabolic disease. The brown adipocytes can provide a glucose sink with high mitochondrial and cellular respiration and fatty acid oxidation rates, dissipating energy as heat (uncoupled oxidative phosphorylation). The subject metabolic rate can be enhanced, and a decrease in body weight can be induced. Induction of the appearance or differentiation of brown adipocytes can also yield improvements in insulin sensitivity, blood glucose homeostasis and cardiovascular disease risk factors. Brown adipocytes may further secrete factors that contribute to reaching a healthy energy balance and low body fat levels, increased insulin sensitivity and improved blood glucose homeostasis or cardiovascular health.

[0041] Accordingly, in some embodiments the agents disclosed herein, or combinations thereof, can be used for treatment of a subject, including a human subject. In some aspects, these agents may promote the differentiation of BAT progenitor cells into brown adipocytes. In other aspects these agents may induce the expression of UCP1, FABP4 (aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in BAT progenitor cells in vitro, in vivo, or both.

[0042] In some aspects the treated metabolic disease may be obesity, type II diabetes, insulin resistance, hyperinsulinemia, hypertension, hyperlipidemia, hepatosteatosis, fatty liver, non-alcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome, acanthosis nigricans, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Laurence-Moon syndrome, Prader-Willi syndrome, neurodegenerative diseases, and Alzheimer's disease.

[0043] In other embodiments, agents may be used to activate isolated, autologous BAT progenitor cells that are then used for treatment of a subject, including a human subject.

Identification of Molecular Pathways

[0044] Gene chip studies were performed to identify molecular pathways that play a role in the differentiation of CD31- progenitor cells into brown adipocytes and/or the induction of the expression of UCP1. CD31- cells were isolated from human skeletal muscle biopsies as described previously in WO2013071063 which is incorporated herein by reference, and were used in two studies: (1) cAMP study: CD31- cells were differentiated as described in WO2013071063 and incorporated herein by reference (Control) plus addition of vehicle (Control 1 sample) or cAMP (cAMP sample); and (2) Rosiglitazone study: CD31- cells were differentiated as described previously in WO2013071063 except that rosiglitazone was omitted from the adipogenic medium (Control 2 sample). Rosiglitazone was added only to the second sample (Rosiglitazone sample) in this study. As discussed above, these agents have been shown to promote the differentiation of CD31- cells into brown adipocytes and the expression of UCP1.

[0045] Total RNA was purified from these samples, and transcriptional profiles were assessed with Illumina Human WG-6 BeadChip (Expression Analysis, Inc., Durham, NC). Results were analyzed with Ingenuity Pathway Analysis 7.0 (trial version). These results were used to determine what molecular pathways are involved in the differentiation of CD31- cells into brown adipocytes, and, more importantly, what molecular targets can be used for the development of agents that promote the appearance of brown adipocytes and the expression of UCP1.

[0046] Based on this work, the following mechanisms and agents were found to promote brown adipocyte development from BAT progenitor cells: a PPAR γ ligand (e.g., rosiglitazone), a PDE3 inhibitor (e.g., siguazodan), a PDE4 inhibitor (e.g., rolipram), BMP7 (bone morphogenetic protein 7, UniProt P18075), BMP5 (bone morphogenetic protein 5, UniProt P22003), BMP6 (bone morphogenetic protein 6, UniProt P22004), FGF7 (fibroblast growth factor-7, KGF, keratinocyte growth factor), FGF 10 (fibroblast growth factor-10, KGF-2, keratinocyte growth factor-2), BNP (b-type natriuretic peptide), FGF13 (fibroblast growth factor-13), BDNF (brain-derived neurotrophic factor), a stimulator of soluble guanylate cyclase (sGC) (e.g., riociguat (BAY 63-2521, CAS 625115-55-1), a TRPM8 (CMRI) ligand (e.g., menthol, icilin), Platelet-derived growth factor receptor-like protein (PDGFR α , UniProt Q15198), Vascular endothelial growth factor D (VEGF-D, FIGF, UniProt 043915), CYTL1 (cytokine-like protein 1, UniProt Q9NRR1), SCG2 (secretogranin-2, UniProt P13521), NPTX2 (neuronal pentraxin-2, UniProt P47972), OLFML2B (olfactomedin-like protein 2B, UniProt Q68BL8), TFPI2 (tissue factor pathway inhibitor 2, UniProt P48307), IFNE (interferon epsilon, UniProt Q86WN2), and a Prostaglandin F2- α receptor (PTGFR, prostanoic acid receptor, UniProt P43088) ligand such as prostaglandin F2. Still other mechanisms/agents that were found to promote brown adipocyte development from BAT progenitor cells based on gene chip data include: a peptide derived from the Pituitary adenylate cyclase-activating polypeptide (PACAP, ADCYAP1, UniProt P18509) gene such as PACAP Propeptide of 55 aa (aa 25-79) (200 nM-2 μ M), CNTF (ciliary neurotrophic factor), Interleukin-6 (IL-6, UniProt P05231), Interleukin-15 (IL-15, UniProt P40933), CXCL12 (chemokine (C-X-C motif) ligand 12), stromal cell-

derived factor 1 (SDF1, UniProt P48061) isoform SDF-1g/UniProt P48061-3), and/or a ligand of Atypical chemokine receptor 3 (ACKR3, CMKOR1, CXCR7, GPR159, RDC1, UniProt P25106) such as SDF1 (CXCL12).

Screening of potential modulators of human UCP1 mRNA

[0047] CD31- cells can be used as a tool to identify agents (e.g., compounds, proteins, biologicals, and the like) that induce the differentiation of these cells into brown adipocytes or modulate the expression of UCP1. For example, an RT-PCR based approach can be used to measure UCP1 mRNA levels which may be affected by certain agents.

[0048] This allows the identification of agents that can enhance the differentiation of CD31- cells into brown adipocytes and/or the expression of UCP1 by enhancing the transcription of the UCP1 gene and/or by stabilizing the UCP1 transcript.

[0049] For example, a PPAR γ ligand like rosiglitazone can be used to promote the differentiation of CD31-progenitor cells into brown adipocytes (FIGS. 1-19, 20A, 21, 22). Another example is the use of the recombinant protein, human BMP-7 (FIGS. 1-19, 21).

[0050] A robust method, previously disclosed in WO2013071063 and incorporated herein by reference, was used for detection of CD31- cell differentiation into brown adipocytes by simultaneously quantifying mRNA species corresponding to the brown adipocyte marker UCP1, the adipocyte marker PPAR γ 2, and the "housekeeping" gene cyclophilin A which was used as the internal control.

[0051] This method permits analysis of a large number of samples to identify agents that enhance the differentiation of CD31- cells into brown adipocytes. When differentiated into brown adipocytes CD31-cells express much higher levels of UCP1 and PPAR γ 2 mRNA for a given level of cyclophilin A. UCP1 and PPAR γ 2 mRNA levels normalized to cyclophilin A mRNA levels give an indication of the level of differentiation of the CD31- cells into brown adipocytes, independent of the total number of cells in the sample.

[0052] Quantification of UCP1, PPAR γ 2 and cyclophilin A mRNA by multiplexed TaqMan real-time PCR was thus used to quantify differentiation of the CD31- cells into brown adipocytes.

[0053] Using this method the following agents were identified or confirmed to promote the differentiation of BAT progenitor cells into brown adipocytes and/or induce the expression of UCP1, FABP4 (aP2), PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in BAT progenitor cells in vitro, in vivo, or both: a PPAR γ ligand like rosiglitazone, a PDE3 inhibitor like siguazodan, a PDE4 inhibitor like rolipram, a derivative of prostaglandin F2 (PGF2) like 9 β ,11 α -prostaglandin F2, a peptide derived from the Pituitary adenylate cyclase-activating polypeptide (PACAP, ADCYAP1, UniProt P18509) gene like the PACAP Propeptide of 55 aa (aa 25-79), BDNF (brain-derived neurotrophic factor), a TGR5 agonist like oleanolic acid, BMP-7, kaempferol (KMP, CAS number 520-18-3), a stimulator of soluble guanylate cyclase (sGC) like riociguat (BAY 63-2521, CAS 625115-55-1), FGF7 (fibroblast growth factor-7, KGF, keratinocyte growth factor), FGF10 (fibroblast growth factor-10, KGF-2, keratinocyte growth factor-2), BNP (b-type natriuretic peptide), a TRPM8 (CMRI) ligand like menthol or icilin, bombesin, CNTF (ciliary neurotrophic factor), interleukin-6 (IL-6), orexin B, SDF-1 γ (CXCL12), and FGF13 (fibroblast growth factor-13).

[0054] Except otherwise indicated, all organic and inorganic chemicals of analytical or molecular biology grade were purchased from Sigma Chemical Co. (St Louis, MI), Life Technologies (Grand Island, NY), GenScript, Prospec, LifeTein, AnaSpec. Rosiglitazone was purchased from Cayman Chemical (# 71742) and recombinant human BMP7 (rhBMP7) was from R&D Systems (100 μ g/ml, 6.3 μ M, # 354-BP-010).

Cell culture

[0055] Cells were seeded at 10,000 per cm² in 0.2% gelatin coated plates (48-well tissue culture, Chemglass # CLS-3500-048), cultured until confluency (2-4 days) at 37°C in Endothelial cell growth medium-2 (EGM2) (BulletKit growth medium, Lonza # CC-3162) and until differentiation (10-16 more days). After 2 or 3 days in EGM2 medium the cells were induced to differentiate by replacing the medium with an adipogenic medium, which is a modification of the adipogenic medium described by Rodriguez et al. [21] and may or may not contain a differentiation inducing agent (e.g., PPAR γ agonist). The MDM described above contains: DMEM/Ham's F-12 50/50 Mix (3.151 g/l, 17.5 mM D-glucose, 3.651g/l L-glutamine) (Cellgro # 10-090-CV), 5 μ g/ml (0.86 μ M) insulin, 1 μ M dexamethasone, 100 μ M 3-isobutyl-1-methylxanthine, 0.2 nM 3,3',5-triiodo-L-thyronine, 10 μ g/ml (127 nM) transferrin, and 1% penicillin-streptomycin. If rosiglitazone is used as a differentiation-inducing agent, it can be supplied at 1 μ M or any other concentration sufficient to induce differentiation of BAT progenitor cells into adipocytes.

[0056] For cell expansion studies, confluent cells grown in EGM2 medium only were detached by treatment with trypsin-EDTA for 3-5 min at 37°C, and then split 1:3 or 1:4 and cultured as described above.

Quantification of UCP1 and PPAR γ 2 mRNA by quantitative reverse transcription, real-time PCR

[0057] Total RNA was prepared from cells using PureLink RNA Isolation Kit (Invitrogen # 12183-016) First strand

cDNA were synthesized using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) and random primers.

[0058] Quantitative real-time PCR was performed using an Applied Biosystems StepOnePlus™ instrument, TaqMan Gene Expression Master Mix (Applied Biosystems # 4369016), and custom TaqMan gene expression probes and primers for human uncoupling protein-1 "UCP1" (GenBank NM_021833) and for human peptidylprolyl isomerase A "cyclophilin A" (GenBank NM_021130). Custom TaqMan Gene Expression reagents were also developed for simultaneous measurement of peroxisome proliferator-activated receptor gamma, transcript variant 2 (PPARγ2) (GenBank NM_015869) in a multiplexed fashion (with UCP1 and cyclophilin A): UCP1 FAM-MGB probe: TCA AGG GGT TGG TAC CTT CC (SEQ ID NO.: 1), sense primer: CAC TAA CGA AGG ACC AAC GG (SEQ ID NO.: 2), and antisense primer: TTC CAG GAT CCA AGT CGC AA (SEQ ID NO.: 3). Cyclophilin A NED-MGB probe: ACT GCC AAG ACT GAG TGG TT (SEQ ID NO.: 4), sense primer: CAA ATG CTG GAC CCA ACA CA (SEQ ID NO.: 5), and antisense primer: TCA CTT TGC CAA ACA CCA CA (SEQ ID NO.: 6). PPARγ2 VIC-MGB probe: TCA CAA GAA ATG ACC ATG GTT G (SEQ ID NO.: 7), sense primer: AGC GAT TCC TTC ACT GAT ACA C (SEQ ID NO.: 8), and antisense primer: CCA GAA TGG CAT CTC TGT GT (SEQ ID NO.: 9).

[0059] Cyclophilin A was used as a control to account for any variations due to the efficiency of reverse transcription. Arbitrary units were determined by normalizing target mRNA levels to cyclophilin A mRNA levels (based on Cts).

Statistical analysis

[0060] Data are expressed as means $\pm$ SEM. Significances were evaluated using the unpaired Student's t-test. Significances were set at $p < 0.05$.

Pictures for cell morphology

[0061] Pictures of cells were taken using a hand-held digital camera (Nikon Coolpix 950) and inverted microscope (Nikon TMS) used for cell culture observations; images were optimized using Adobe Photoshop Elements 8 functions for Auto Contrast and Auto Levels.

[0062] The section headings and subheadings used in this specification are for organizational purposes only and are not to be construed as limiting the subject matter described in any way. Further, while the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents as will be appreciated by those of skill in the art.

Quantification of UCP1 protein

[0063] Differentiation of brown adipocyte progenitors into brown adipocytes can be detected through quantification of UCP1 protein by immunohistochemistry (IHC).

[0064] Culturing and differentiation of CD31- cells into brown adipocytes were performed using adipogenic differentiation medium lacking (Minimal Differentiation Medium, MDM) or containing 1 μ M rosiglitazone (Reference Differentiation Medium, RDM). After 15 days of differentiation cells were fixed with 4% Paraformaldehyde PBS pH 7.4, and incubated with a UCP1 antibody (Abcam ab23841) and Alexafluor 488 goat anti-rabbit antibody to quantify relative UCP1 levels (green) according to standard protocols. Prior to fixation of cells, nuclei were labeled with 5 μ M DAPI (blue) for 10 minutes. Each treatment condition was evaluated in triplicate in a 96-well plate corresponding to approximately 360-480 cells for each data point in total. The InCell 1000 Developer Toolbox software was used to develop an automated cell detection script to measure UCP1 signal intensity, using the nuclei and cytoplasm detection algorithms. As a readout, total intensity of UCP1 signal within the cell was used, normalized to cell number.

[0065] In some embodiments, agents or combinations thereof that were identified using this technique include Famotidine, Tiapride hydrochloride, Guanfacine hydrochloride, Reserpine, Minoxidil, Spiperone, Diflunisal, Syrosingopine, Probenecid, Metformin, Thiethylperazine, Colchicine, and Felodipine.

Detection of brown adipocyte differentiation

[0066] BODIPY fluorescent dye-labeled neutral lipids become incorporated in cytoplasmic lipid droplets allowing analysis of cellular fatty acid uptake and adipocyte differentiation by fluorescent cellular imaging. Cells are incubated with C1-BODIPY® 500/510 C12 (Molecular Probes # D-3823) for 3 to 6 hours before imaging on a microplate-based high-throughput, high-content, brightfield and fluorescence cellular imager and analyzer (Cytellect Celigo® or GE Healthcare IN Cell Analyzer).

OTHER EMBODIMENTS

[0067] Various aspects of the present disclosure may be used alone, in combination, or in a variety of arrangements not specifically discussed in the embodiments described in the foregoing and is therefore not limited in its application to the details and arrangement of components set forth in the foregoing description or illustrated in the drawings. For example, aspects described in one embodiment may be combined in any manner with aspects described in other embodiments.

[0068] Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[0069] Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having," "containing," "involving," and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

[0070] The present disclosure provides among other things novel compositions capable of recruiting brown adipocytes in vitro and in vivo. While specific embodiments of the subject disclosure have been discussed, the above specification is illustrative and not restrictive. Many variations of the disclosure will become apparent to those skilled in the art upon review of this specification. The full scope of the disclosure should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

INCORPORATION BY REFERENCE

[0071] All publications, patents and patent applications referenced in this specification are incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically indicated to be so incorporated by reference.

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ASPECTS OF THE DISCLOSURE

[0073]

Aspect 1. Use of an agent for recruiting brown adipocytes from BAT progenitor cells isolated from human skeletal muscle, wherein the agent is selected from one or more of:

an antihistamine such as Famotidine;

an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine or Spiperone;
 a ligand of tubulin such as Colchicine;
 a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 5 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
 a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP
 Propeptide of 55 aa (aa 25-79);
 10 a flavonoid such as kaempferol;
 a fibroblast growth factor (FGF) such as FGF7 or FGF10 or FGF13;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 bombesin;
 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ;
 15 a cyclooxygenase inhibitor such as Diflunisal;
 a biguanide such as Metformin;
 a phosphodiesterase inhibitor such as a PDE3 inhibitor such as siguazodan;
 a stimulator of soluble guanylate cyclase (sGC) such as riociguat;
 b-type natriuretic peptide (BNP);
 20 ciliary neurotrophic factor (CNTF);
 interleukin-6 (IL-6);
 orexin B; and
 an α 2 adrenergic receptor agonist such as Guanfacine hydrochloride.

25 Aspect 2. The use of aspect 1, wherein the agent is selected from one or more of:

an antihistamine such as Famotidine;
 an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine;
 a ligand of tubulin such as Colchicine;
 30 a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
 35 a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP
 Propeptide of 55 aa (aa 25-79);
 a flavonoid such as kaempferol;
 a fibroblast growth factor (FGF) such as FGF7 or FGF10;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 40 bombesin;
 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ; and
 a cyclooxygenase inhibitor such as Diflunisal.

45 Aspect 3. The use of aspect 1 or 2, wherein the agent is capable of inducing expression of UCP1, FABP4 (aP2),
 PPAR γ 2, mtTFA, PGC-1 α , and/or COX IV in the BAT progenitor cells in vitro, in vivo, or both. Aspect 4. The use of
 aspect 1 or 2, wherein said agent has one or more biological activities selected from the group consisting of:

(a) causing an increase or decrease in one or more of the following: Beta-3 adrenergic receptor (β 3-AR), Solute
 carrier family 2, facilitated glucose transporter member 4 (SLC2A4), Elongation of very long chain fatty acids
 50 protein 3 (ELOVL3), CD36 antigen, Type II iodothyronine deiodinase (DIO2), BMP5, BMP6, FGF7, FGF10,
 FGF13, FGF21, Fatty acid binding protein 7 (FABP7), CXCL12, Atypical chemokine receptor 3 (ACKR3), Insulin-
 like growth factor-binding protein 4 (IGFBP4), Pituitary adenylate cyclase-activating polypeptide (PACAP), Ade-
 nylate cyclase 4 (ADCY4), Cell death activator CIDE-A (CIDEA), secreted frizzled-related protein 1 (SRFP1),
 SRFP2, brain-derived neurotrophic factor (BDNF), Vascular endothelial growth factor D (VEGF-D), Transforming
 55 growth factor beta-2 (TGFB2), cAMP-specific 3',5'-cyclic phosphodiesterase 4B (PDE4B), cAMP-specific 3',5'-
 cyclic phosphodiesterase 4D (PDE4D), High affinity cAMP-specific 3',5'-cyclic phosphodiesterase 7A (PDE7A),
 and cAMP-specific 3',5'-cyclic phosphodiesterase 7B (PDE7B);
 (b) causing an increase in thermogenesis in brown adipose tissue and/or skeletal muscle tissue;

- (c) causing an increase in insulin sensitivity of skeletal muscle, white adipose tissue, or liver;
- (d) causing an increase in glucose tolerance;
- (e) causing an increase in basal respiration, maximal respiration rate, or uncoupled respiration;
- (f) causing an increase in metabolic rate; and
- (g) causing a decrease in hepatosteatosis.

Aspect 5. The use of aspect 4, wherein the agent causes an increase or decrease in one or more of the following: Beta-3 adrenergic receptor (β 3-AR), Solute carrier family 2, facilitated glucose transporter member 4 (SLC2A4), Elongation of very long chain fatty acids protein 3 (ELOVL3), CD36 antigen, and Type II iodothyronine deiodinase (DIO2).

Aspect 6. The use of aspect 1 or 2, wherein the agent is capable of modulating a metabolic response in a subject or preventing or treating a metabolic disorder in a subject.

Aspect 7. The use of aspect 6, wherein the metabolic disorder is one or more of obesity, type II diabetes, insulin resistance, hyperinsulinemia, hypertension, hyperlipidemia, hepatosteatosis, fatty liver, non-alcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome, acanthosis nigricans, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Laurence-Moon syndrome, Prader-Willi syndrome, neurodegenerative diseases, and Alzheimer's disease.

Aspect 8. A method of promoting brown adipogenesis in a subject in need thereof, the method comprising administering to the subject an agent selected from one or more of:

- an antihistamine such as Famotidine;
- an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine or Spiperone;
- a ligand of tubulin such as Colchicine;
- a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
- a potassium channel ligand such as Minoxidil;
- an antagonist of calcium channels such as Felodipine;
- Probenecid;
- a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
- a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP Propeptide of 55 aa (aa 25-79);
- a flavonoid such as kaempferol;
- a fibroblast growth factor (FGF) such as FGF7 or FGF10 or FGF13;
- a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
- bombesin;
- stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ;
- a cyclooxygenase inhibitor such as Diflunisal;
- a biguanide such as Metformin;
- a phosphodiesterase inhibitor such as a PDE3 inhibitor such as siguazodan;
- a stimulator of soluble guanylate cyclase (sGC) such as riociguat;
- b-type natriuretic peptide (BNP);
- ciliary neurotrophic factor (CNTF);
- interleukin-6 (IL-6);
- orexin B; and
- an α 2 adrenergic receptor agonist such as Guanfacine hydrochloride.

Aspect 9. The method of aspect 8, wherein the agent is selected from one or more of:

- an antihistamine such as Famotidine;
- an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine;
- a ligand of tubulin such as Colchicine;
- a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
- a potassium channel ligand such as Minoxidil;
- an antagonist of calcium channels such as Felodipine;
- Probenecid;
- a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
- a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP Propeptide of 55 aa (aa 25-79);
- a flavonoid such as kaempferol;

a fibroblast growth factor (FGF) such as FGF7 or FGF10;
a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
bombesin;
stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ; and
a cyclooxygenase inhibitor such as Diflunisal.

Aspect 10. The method of aspect 8 or 9, further comprising modulating a metabolic response in the subject and/or preventing or treating a metabolic disorder in the subject.

Aspect 11. The method of aspect 10 wherein the metabolic disorder is one or more of obesity, type II diabetes, insulin resistance, hyperinsulinemia, hypertension, hyperlipidemia, hepatosteatosis, fatty liver, non-alcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome, acanthosis nigricans, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Laurence-Moon syndrome, Prader-Willi syndrome, neurodegenerative diseases, and Alzheimer's disease.

Aspect 12. The method of aspect 8, further comprising contacting a cell of the subject with the agent.

Aspect 13. The method of aspect 12, further comprising transplantation of said cell into the subject after said contacting step.

Aspect 14. The method of aspect 12 wherein the cell is a BAT progenitor cell isolated from human skeletal muscle.

Aspect 15. The method of aspect 14 wherein the cell is positive for CD34.

Aspect 16. The method of aspect 14 wherein the cell is negative for CD31.

Aspect 17. A method of preventing hypothermia in a subject, comprising administering to the subject an agent selected from one or more of:

an antihistamine such as Famotidine;
an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine or Spiperone;
a ligand of tubulin such as Colchicine;
a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
a potassium channel ligand such as Minoxidil;
an antagonist of calcium channels such as Felodipine;
Probenecid;
a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP Propeptide of 55 aa (aa 25-79);
a flavonoid such as kaempferol;
a fibroblast growth factor (FGF) such as FGF7 or FGF10 or FGF13;
a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
bombesin;
stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ;
a cyclooxygenase inhibitor such as Diflunisal;
a biguanide such as Metformin;
a phosphodiesterase inhibitor such as a PDE3 inhibitor such as siguazodan;
a stimulator of soluble guanylate cyclase (sGC) such as riociguat;
b-type natriuretic peptide (BNP);
ciliary neurotrophic factor (CNTF);
interleukin-6 (IL-6);
orexin B; and
an α 2 adrenergic receptor agonist such as Guanfacine hydrochloride.

Aspect 18. The method of aspect 17, wherein the agent is selected from one or more of:

an antihistamine such as Famotidine;
an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine;
a ligand of tubulin such as Colchicine;
a Rauwolfia alkaloid or derivative such as Reserpine or Syrosingopine;
a potassium channel ligand such as Minoxidil;
an antagonist of calcium channels such as Felodipine;
Probenecid;
a derivative of prostaglandin F₂ (PGF₂) such as 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
a peptide derived from Pituitary adenylate cyclase-activating polypeptide (PACAP) gene such as the PACAP

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Propeptide of 55 aa (aa 25-79);
a flavonoid such as kaempferol;
a fibroblast growth factor (FGF) such as FGF7 or FGF10;
a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
5 bombesin;
stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ; and
a cyclooxygenase inhibitor such as Diflunisal.

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SEQUENCE LISTING

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Claims

1. Composition comprising an agent for use in the therapy of a metabolic disorder by recruiting brown adipocytes from brown adipose tissue (BAT) progenitor cells isolated from human skeletal muscle, wherein the agent is selected from one or more of:

an antihistamine such as Famotidine;
 an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine or Spiperone;
 a ligand of tubulin such as Colchicine;
 a Rauwolfia alkaloid or derivative selected from Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F₂ (PGF₂) selected from 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
 a peptide fragment of Pituitary adenylate cyclase-activating polypeptide (PACAP) such as the PACAP Propeptide of 55 aa (aa 25-79);
 a flavonoid such as kaempferol;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 bombesin;
 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ;
 a cyclooxygenase inhibitor such as Diflunisal;
 a phosphodiesterase inhibitor such as a PDE3 inhibitor such as siguazodan;
 a stimulator of soluble guanylate cyclase (sGC) such as riociguat;
 b-type natriuretic peptide (BNP);
 ciliary neurotrophic factor (CNTF);
 interleukin-6 (IL-6);
 orexin B; and
 an α 2 adrenergic receptor agonist such as Guanfacine hydrochloride.

2. The composition of claim 1, wherein the agent is selected from one or more of:

an antihistamine such as Famotidine;
 an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine;
 a ligand of tubulin such as Colchicine;
 a Rauwolfia alkaloid or derivative selected from Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F₂ (PGF₂) selected from 9 β ,11 α -PGF₂ or 9 α ,11 β -PGF₂;
 a peptide fragment of Pituitary adenylate cyclase-activating polypeptide (PACAP) such as the PACAP Propeptide of 55 aa (aa 25-79);
 a flavonoid such as kaempferol;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 bombesin;
 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ; and
 a cyclooxygenase inhibitor such as Diflunisal.

3. The composition of claim 1, wherein the agent is selected from one or more of:

Famotidine;
 Tiapride hydrochloride or Thiethylperazine or Spiperone;
 Colchicine;
 Reserpine or Syrosingopine;
 Minoxidil;
 Felodipine;
 Probenecid;
 9 β , 11 α -prostaglandin F2 (PGF2) or 9 α , 11 β -PGF2;
 Pituitary adenylate cyclase-activating polypeptide (PACAP) Propeptide of 55 aa (aa 25-79);
 kaempferol;
 menthol or icilin;
 bombesin;
 stromal cell-derived factor 1 (SDF-1);
 Diflunisal;
 siguazodan;
 riociguat;
 b-type natriuretic peptide (BNP);
 ciliary neurotrophic factor (CNTF);
 interleukin-6 (IL-6);
 orexin B; and
 Guanfacine hydrochloride.

4. The composition of claim 3, wherein the agent is selected from one or more of:

Famotidine;
 Tiapride hydrochloride or Thiethylperazine;
 Colchicine;
 Reserpine or Syrosingopine;
 Minoxidil;
 Felodipine;
 Probenecid;
 9 β , 11 α -prostaglandin F2 (PGF2) or 9 α , 11 β -POPP₂
 Pituitary adenylate cyclase-activating polypeptide (PACAP) Propeptide of 55 aa (aa 25-79);
 kaempferol;
 menthol or icilin;
 bombesin;
 stromal cell-derived factor 1 (SDF-1) isoform SDF-1 γ ; and
 Diflunisal.

5. The composition of any one of claims 1-4, wherein the metabolic disorder is one or more of obesity, type II diabetes, insulin resistance, hyperinsulinemia, hypertension, hyperlipidemia, hepatosteatosi, fatty liver, non-alcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome, acanthosis nigricans, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Laurence-Moon syndrome, Prader-Willi syndrome, neurodegenerative diseases, and Alzheimer's disease.

6. An ex vivo method of promoting brown adipogenesis using an agent selected from one or more of:

an antihistamine such as Famotidine;
 an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine or Spiperone;
 a ligand of tubulin such as Colchicine;
 a Rauwolfia alkaloid or derivative selected from Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F2 (PGF2) selected from 9 β , 11 α -PGF2 or 9 α , 11 β -PGF2;
 a peptide fragment of Pituitary adenylate cyclase-activating polypeptide (PACAP) such as the PACAP Propeptide

of 55 aa (aa 25-79);
 a flavonoid such as kaempferol;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 bombesin;
 5 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ;
 a cyclooxygenase inhibitor such as Diflunisal;
 a phosphodiesterase inhibitor such as a PDE3 inhibitor such as siguazodan;
 a stimulator of soluble guanylate cyclase (sGC) such as riociguat;
 b-type natriuretic peptide (BNP);
 10 ciliary neurotrophic factor (CNTF);
 interleukin-6 (IL-6);
 orexin B; and
 an α 2 adrenergic receptor agonist such as Guanfacine hydrochloride.

7. The method of claim 6, wherein the agent is selected from one or more of:

an antihistamine such as Famotidine;
 an antidopaminergic such as Tiapride hydrochloride or Thiethylperazine;
 a ligand of tubulin such as Colchicine;
 20 a Rauwolfia alkaloid or derivative selected from Reserpine or Syrosingopine;
 a potassium channel ligand such as Minoxidil;
 an antagonist of calcium channels such as Felodipine;
 Probenecid;
 a derivative of prostaglandin F2 (PGF2) selected from 9 β ,11 α -PGF2 or 9 α ,11 β -PGF2;
 25 a peptide fragment of Pituitary adenylate cyclase-activating polypeptide (PACAP) such as the PACAP Propeptide of 55 aa (aa 25-79);
 a flavonoid such as kaempferol;
 a transient receptor potential melastatin 8 (TRPM8) ligand such as menthol or icilin;
 bombesin;
 30 stromal cell-derived factor 1(SDF-1) such as isoform SDF-1 γ ; and
 a cyclooxygenase inhibitor such as Diflunisal.

8. The method of claim 6, wherein the agent is selected from one or more of:

Famotidine;
 Tiapride hydrochloride or Thiethylperazine or Spiperone;
 Colchicine;
 Reserpine or Syrosingopine;
 Minoxidil;
 40 Felodipine;
 Probenecid;
 9 β ,11 α -prostaglandin F2 (PGF2) or 9 α ,11 β -PGF2;
 Pituitary adenylate cyclase-activating polypeptide (PACAP) Propeptide of 55 aa (aa 25-79);
 kaempferol;
 45 menthol or icilin;
 bombesin;
 stromal cell-derived factor 1 (SDF-1);
 Diflunisal;
 siguazodan;
 50 riociguat;
 b-type natriuretic peptide (BNP);
 ciliary neurotrophic factor (CNTF);
 interleukin-6 (IL-6);
 orexin B; and
 55 Guanfacine hydrochloride.

9. The method of claim 8, wherein the agent is selected from one or more of:

Famotidine;
 Tiapride hydrochloride or Thiethylperazine;
 Colchicine;
 Reserpine or Syrosingopine;
 5 Minoxidil;
 Felodipine;
 Probenecid;
 9 β , 11 α -prostaglandin F2 (PGF2) or 9 α , 11 β -PGF2;
 Pituitary adenylate cyclase-activating polypeptide (PACAP) Propeptide of 55 aa (aa 25-79);
 10 kaempferol;
 menthol or icilin;
 bombesin;
 stromal cell-derived factor 1 (SDF-1) isoform SDF-1 γ ; and
 Diflunisal.

15 **10.** The method of any one of claims 6-9, further comprising contacting a cell with the agent.

11. The method of claim 10 wherein the cell is a brown adipose tissue (BAT) progenitor cell isolated from human skeletal muscle.

20 **12.** The method of claim 11 wherein the cell is positive for CD34, and/or wherein the cell is negative for CD31.

FIG. 1

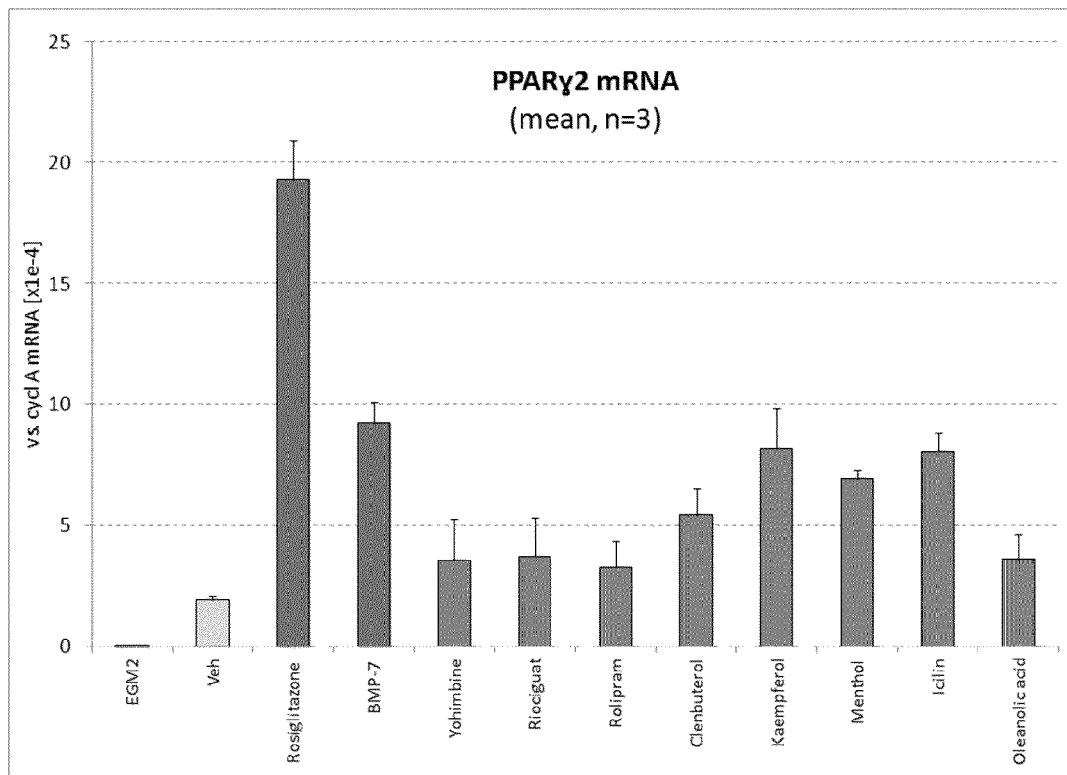


FIG. 2

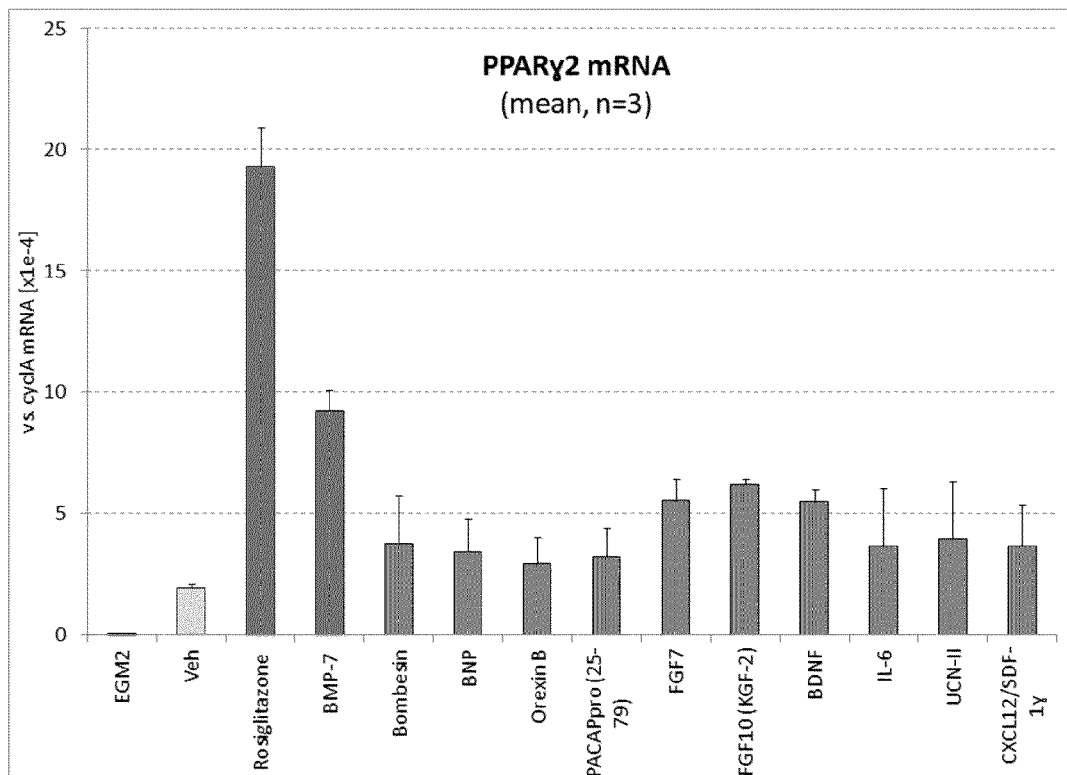


FIG. 3

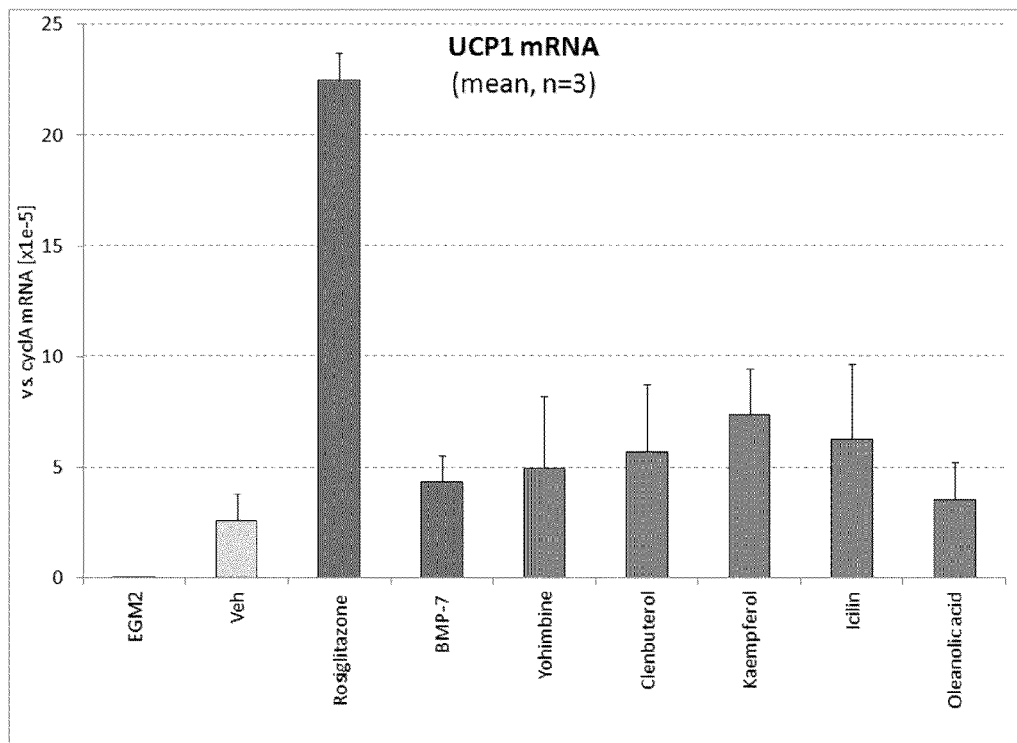


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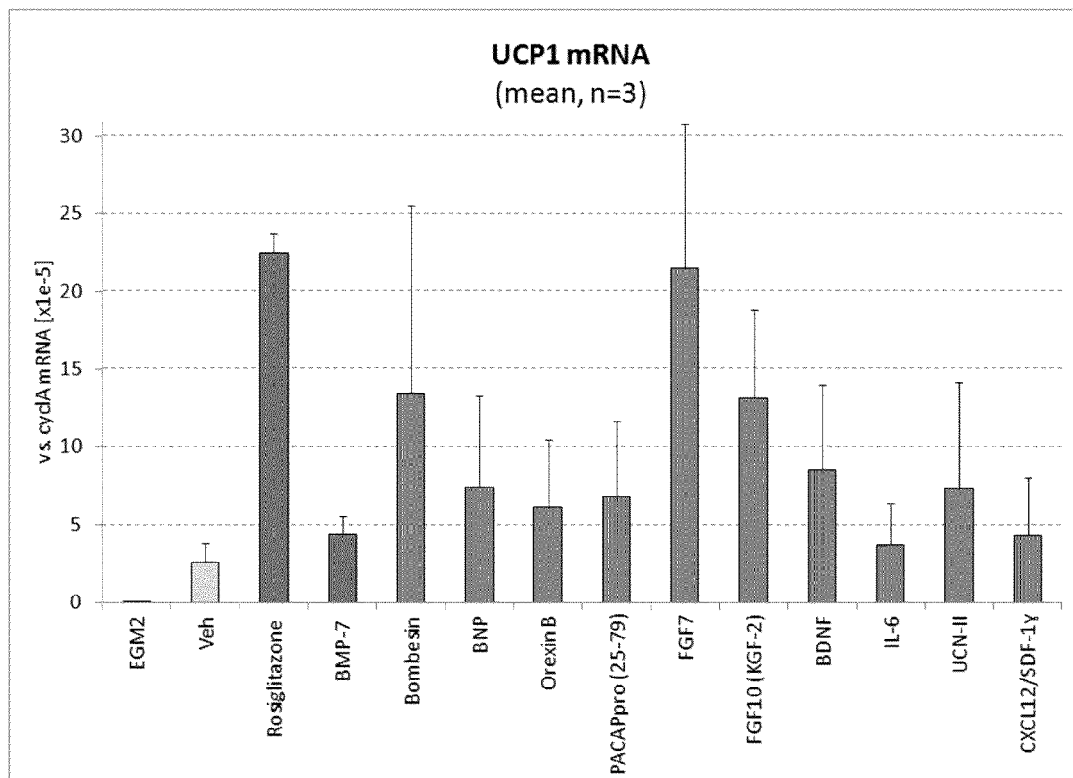


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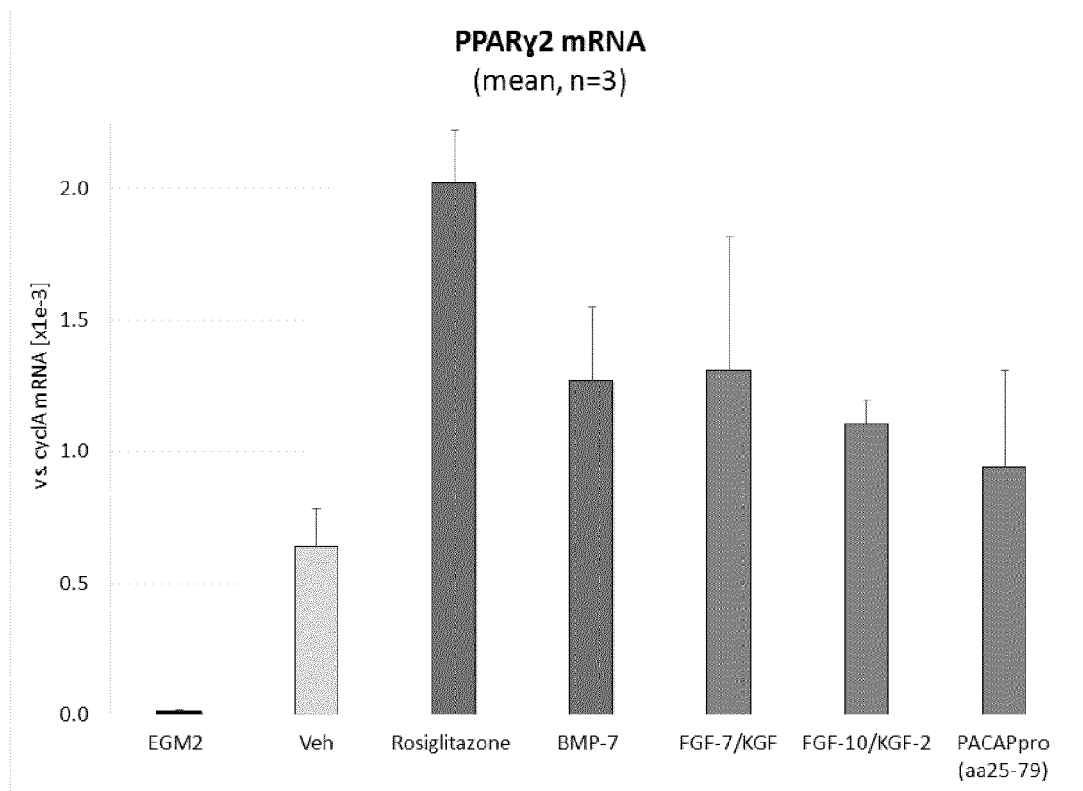


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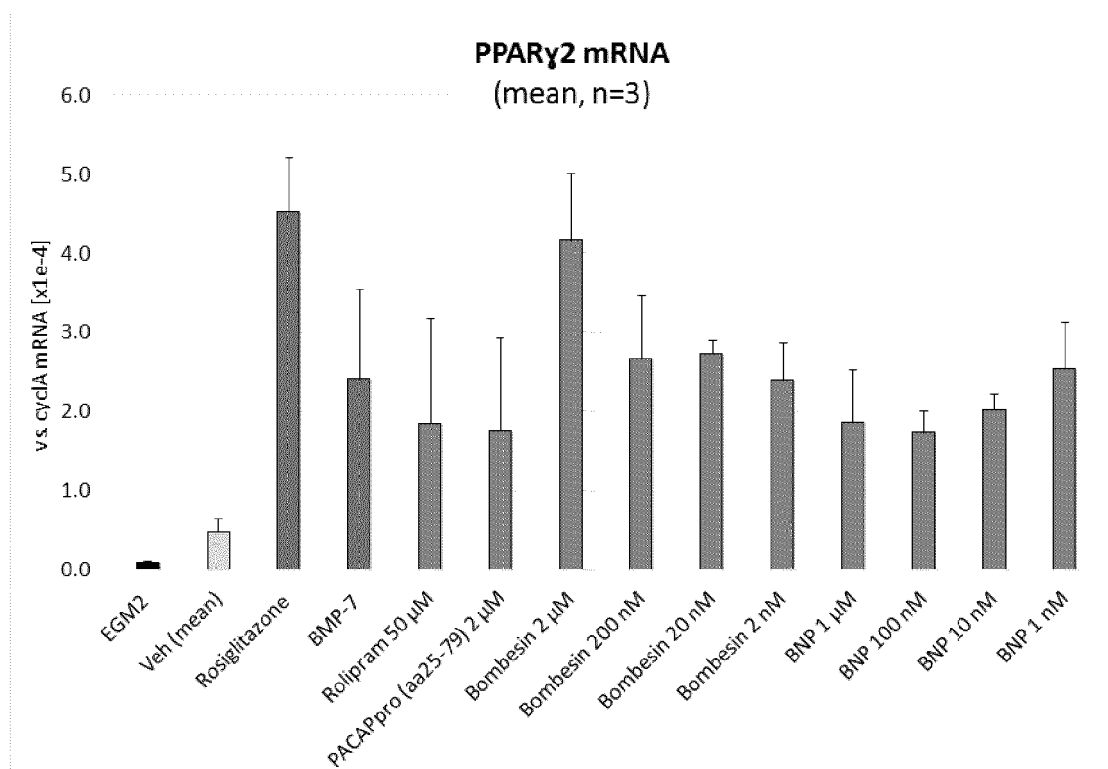


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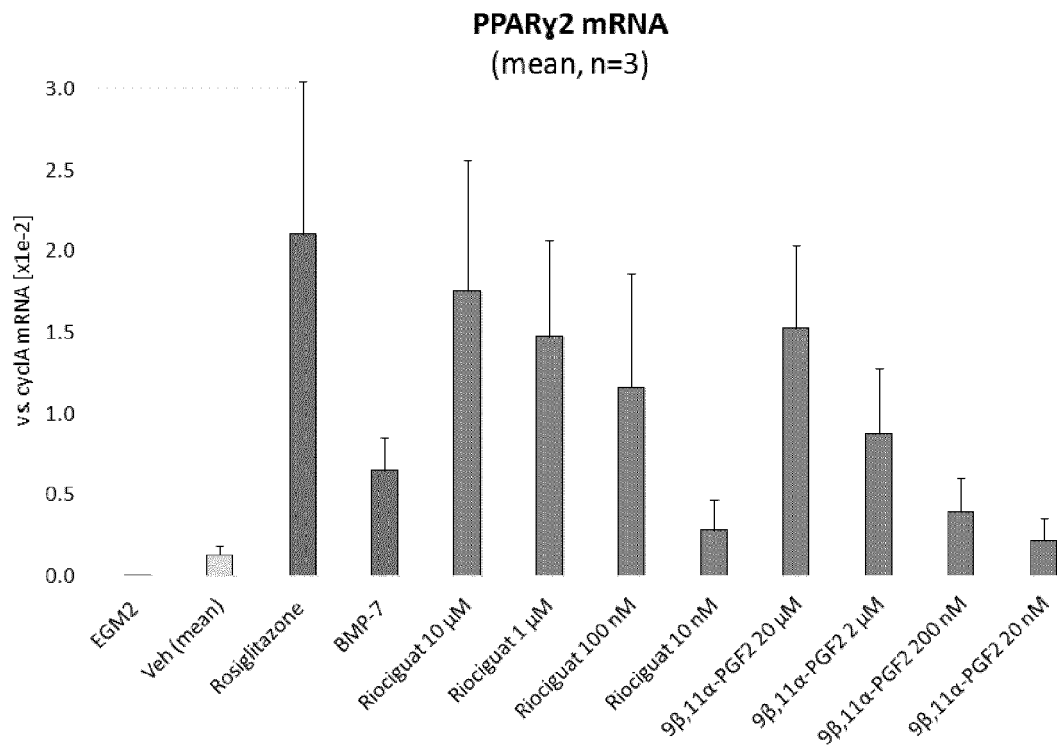


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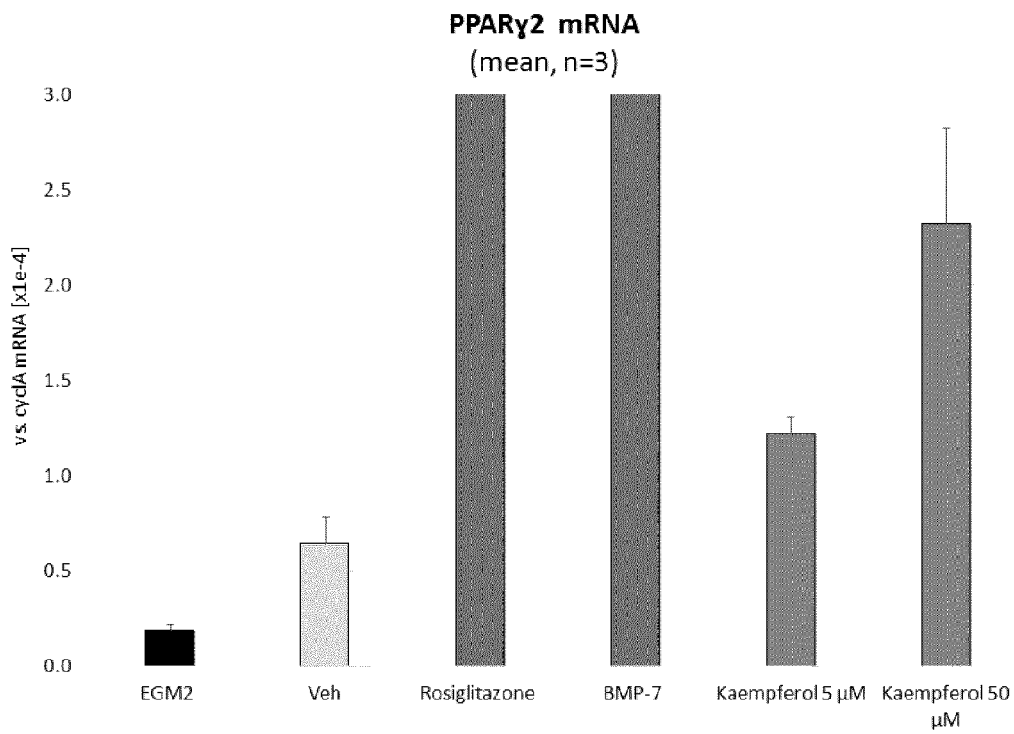


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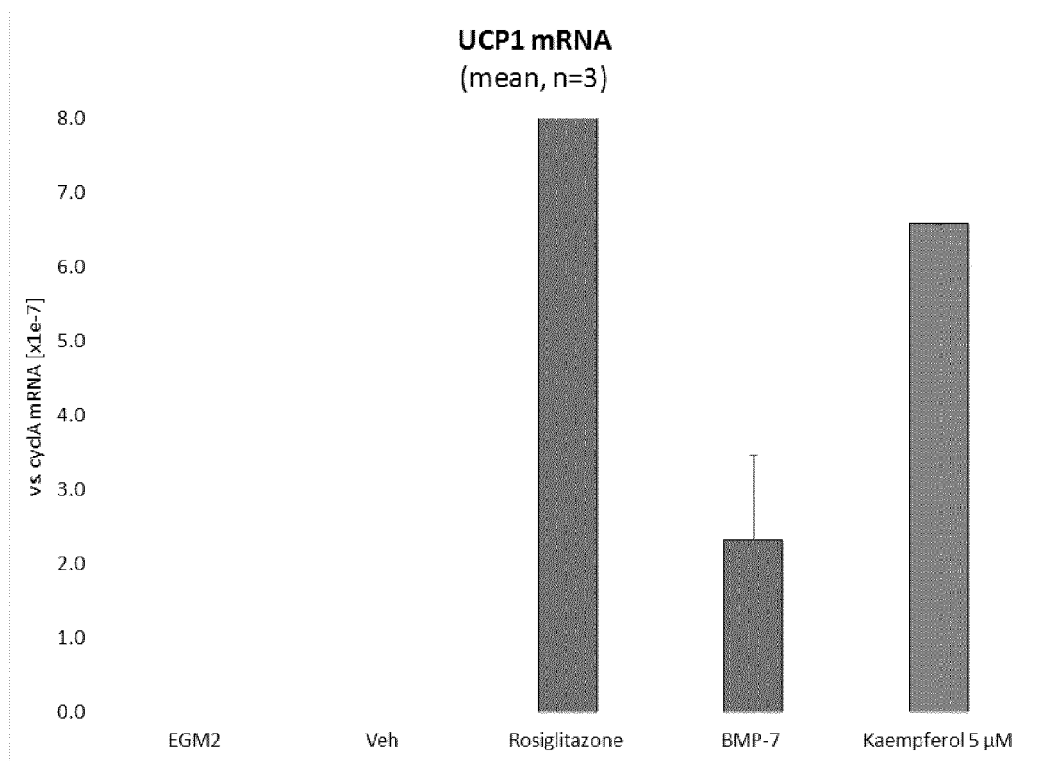


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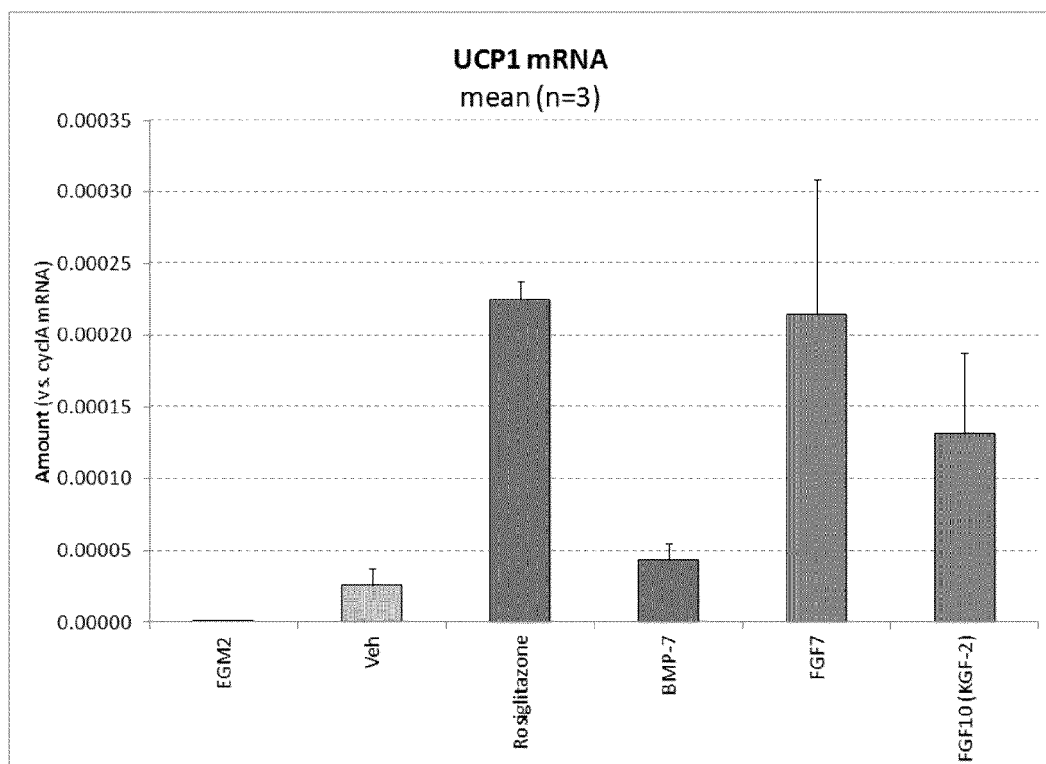


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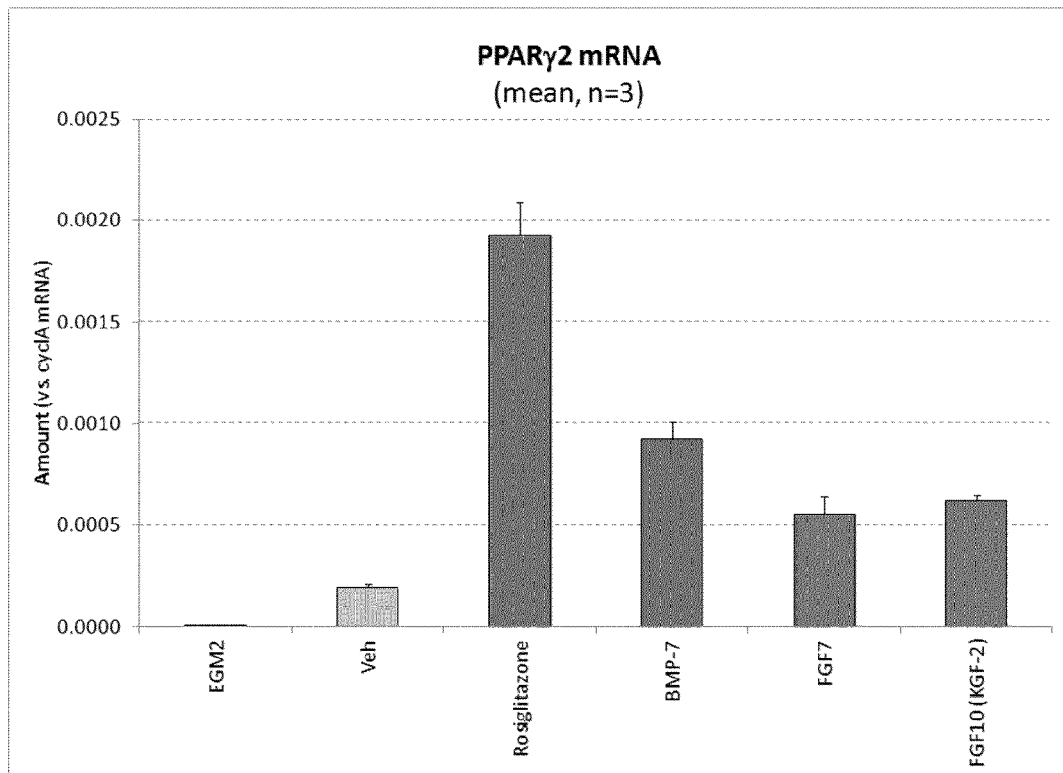


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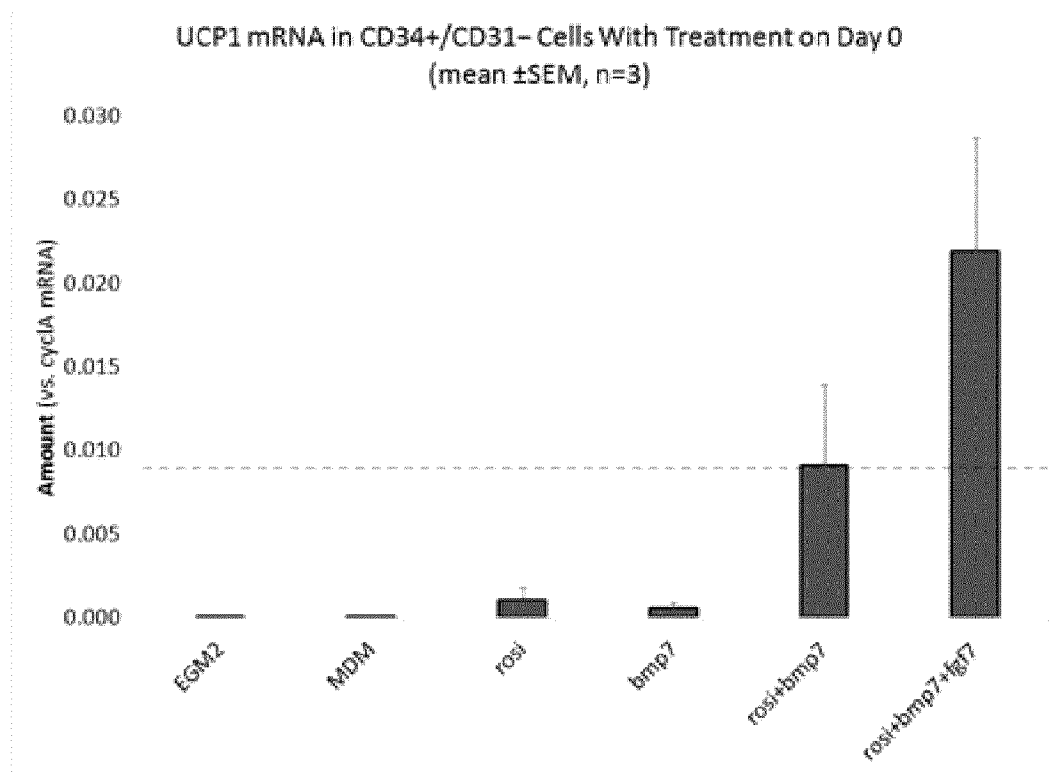


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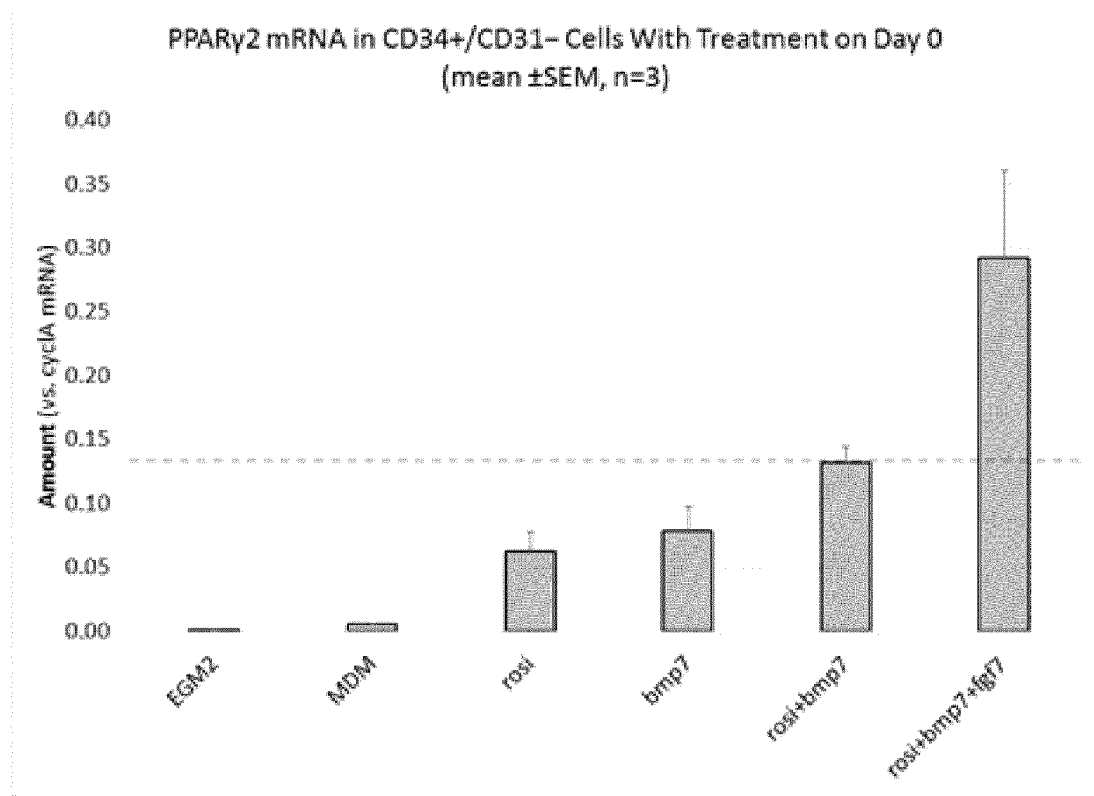


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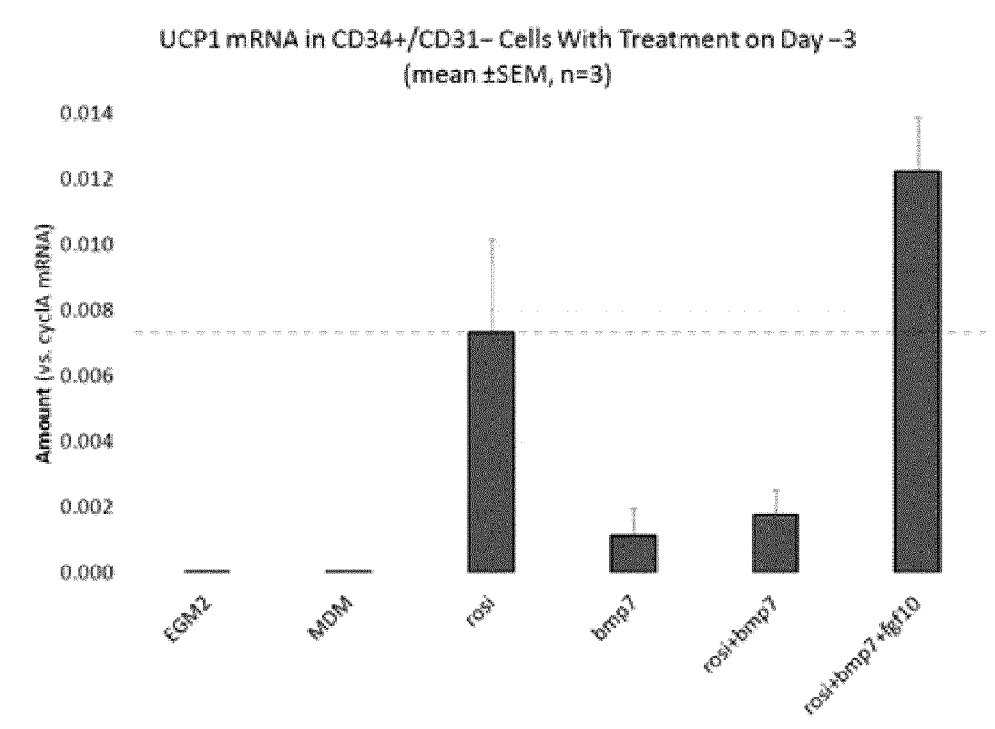


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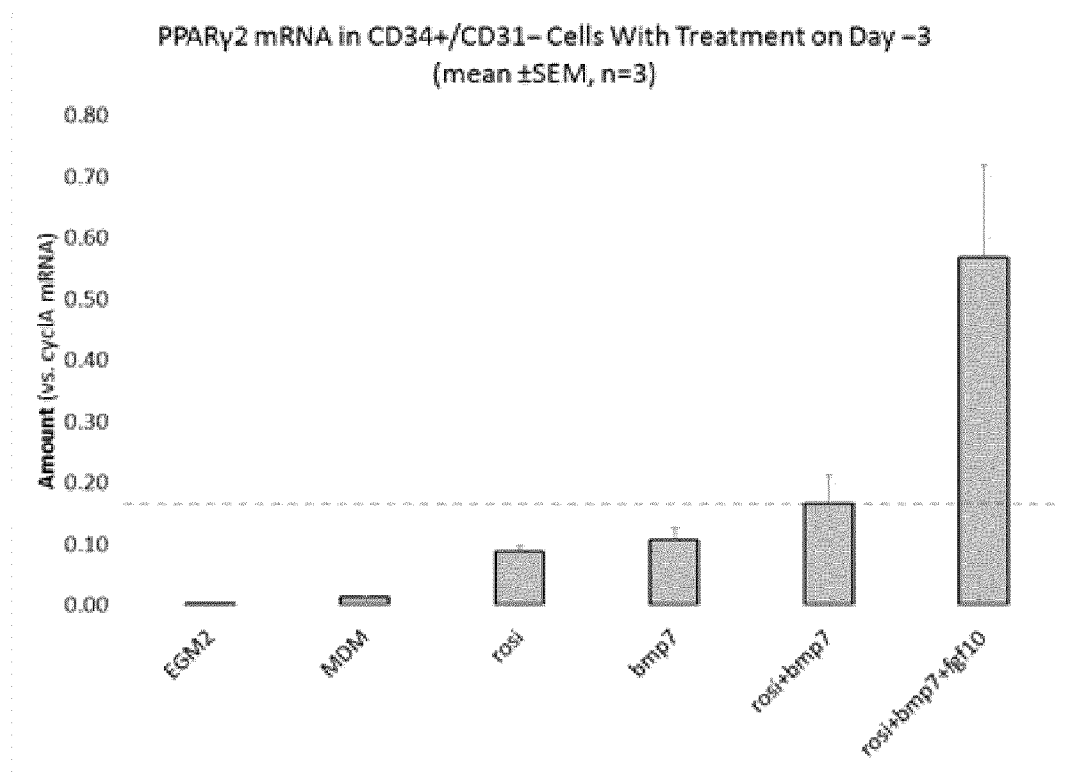


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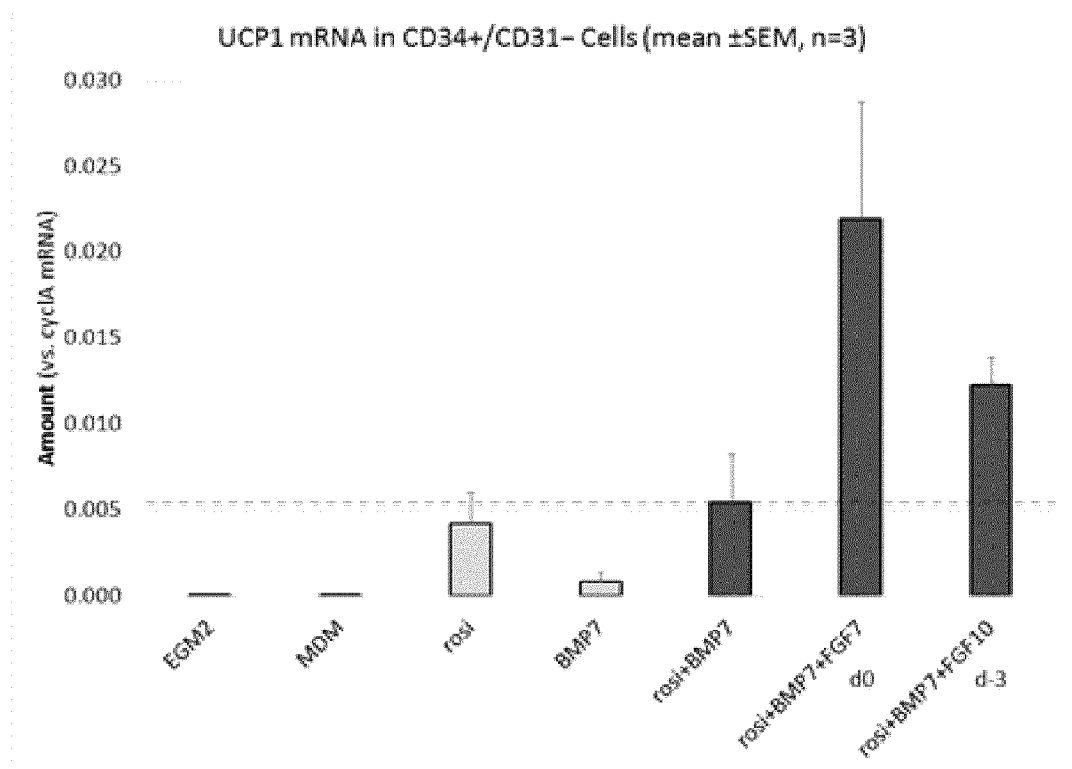


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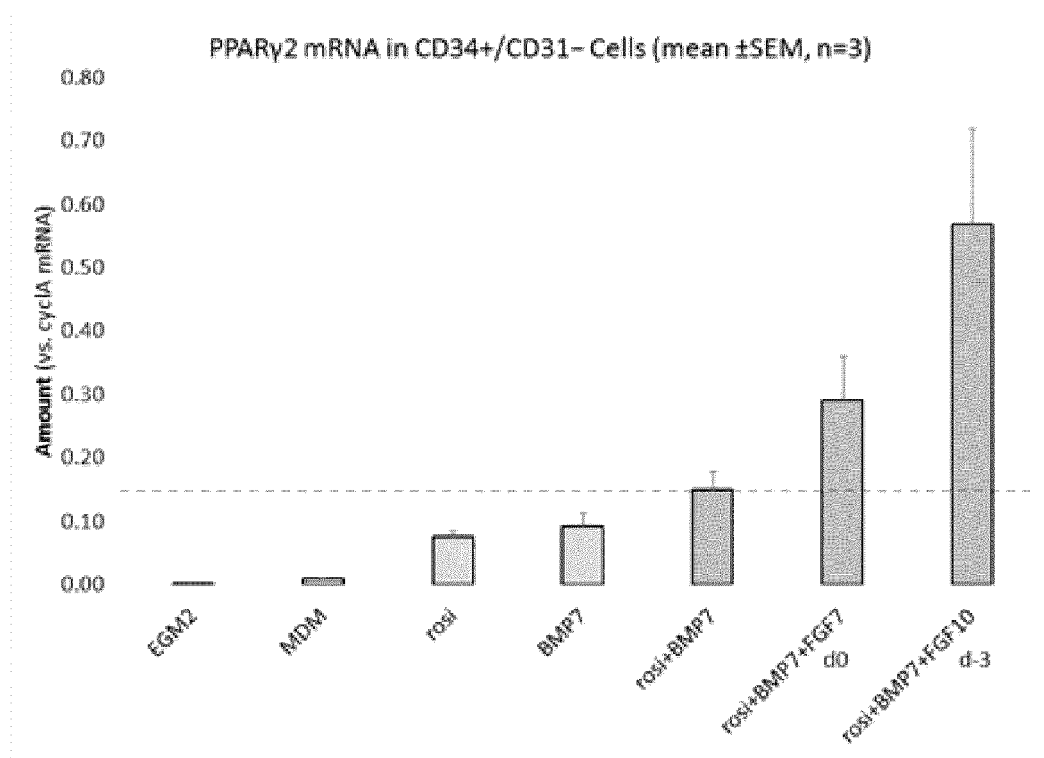


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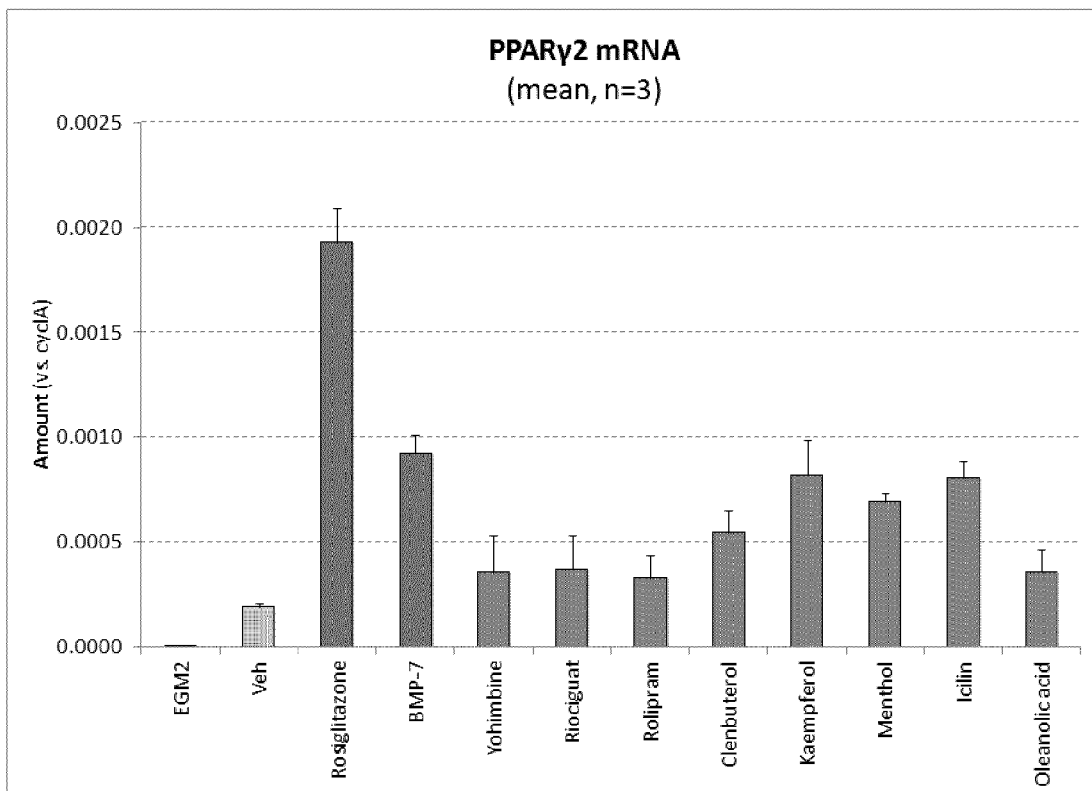


FIG. 19

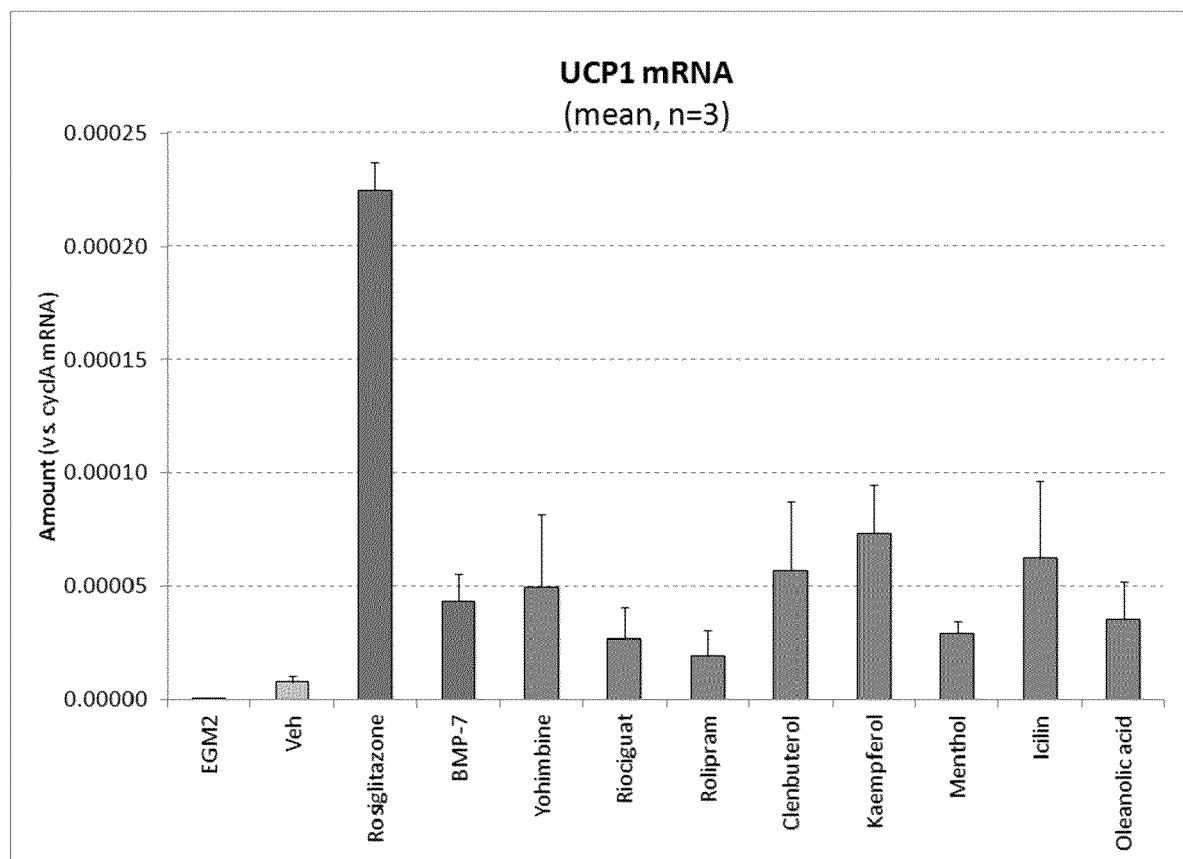


FIG. 20A

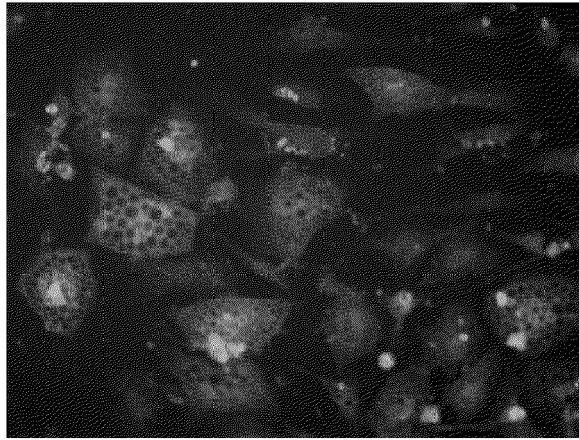


FIG. 20B

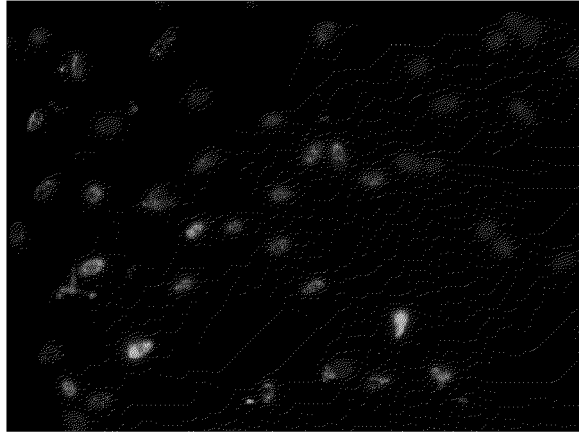


FIG. 20C

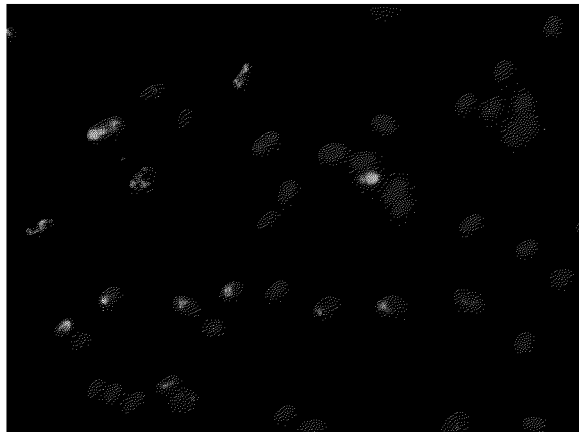


FIG. 21

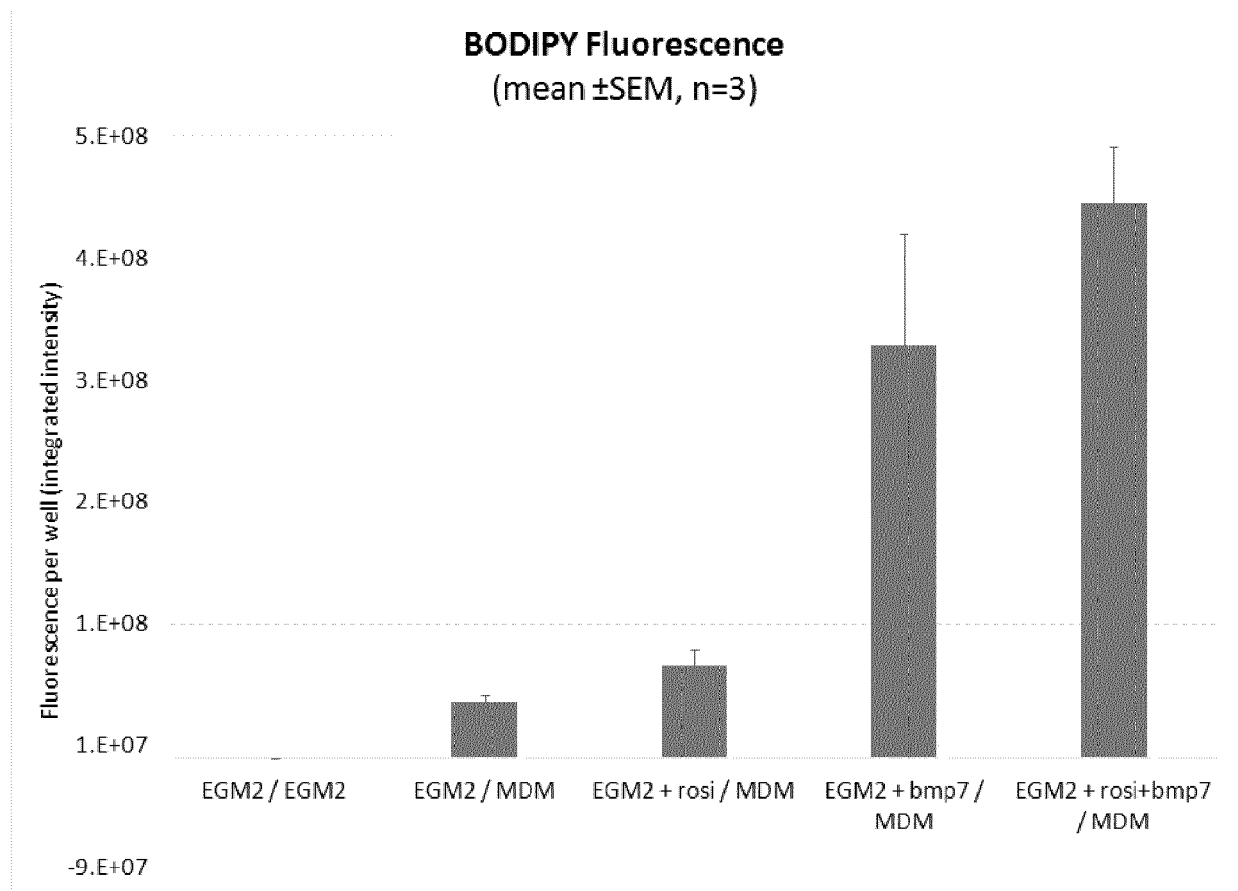


FIG. 22

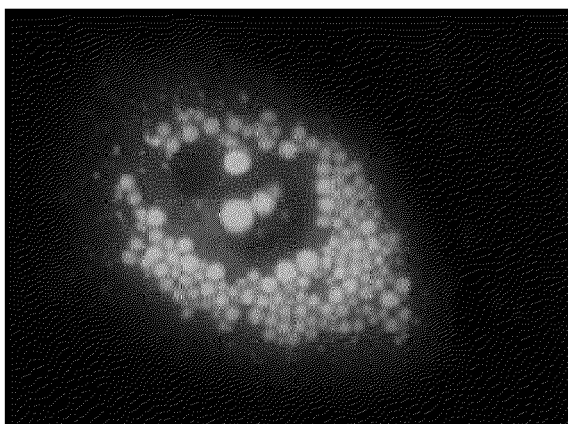


FIG. 23A



FIG. 23B

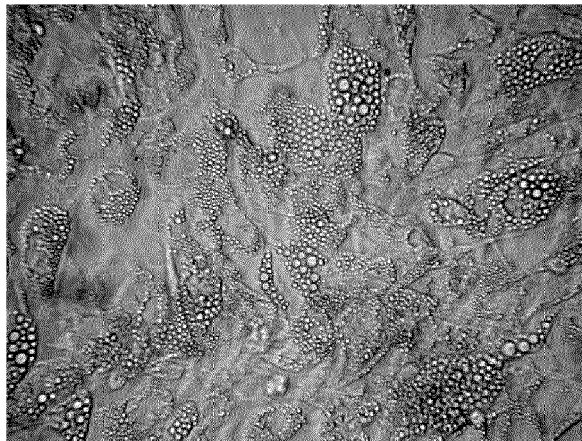


FIG. 23C

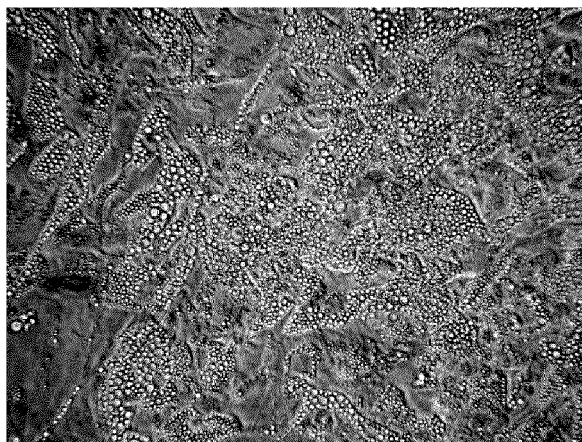


FIG. 23D

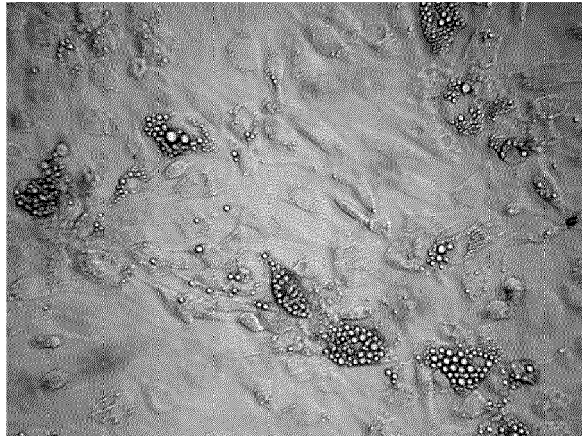


FIG. 23E

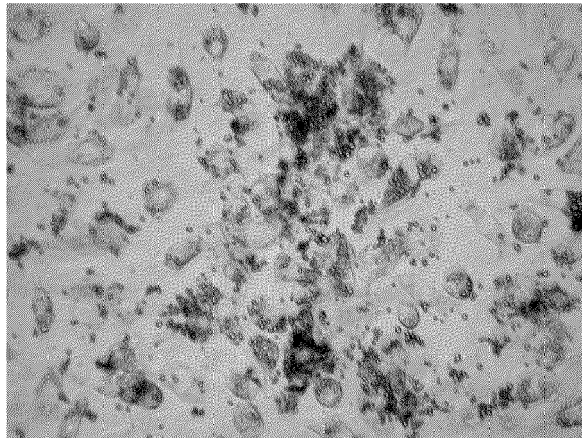


FIG. 23F

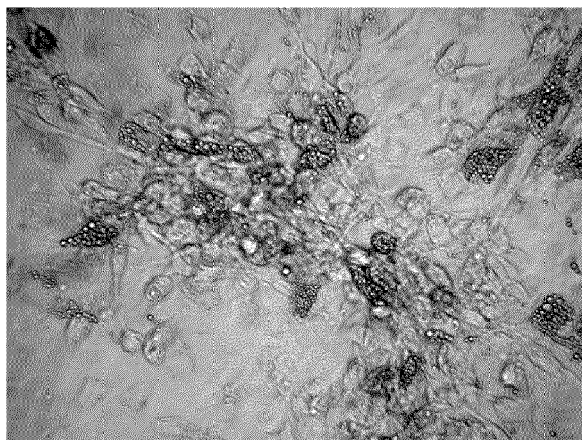


FIG. 23G

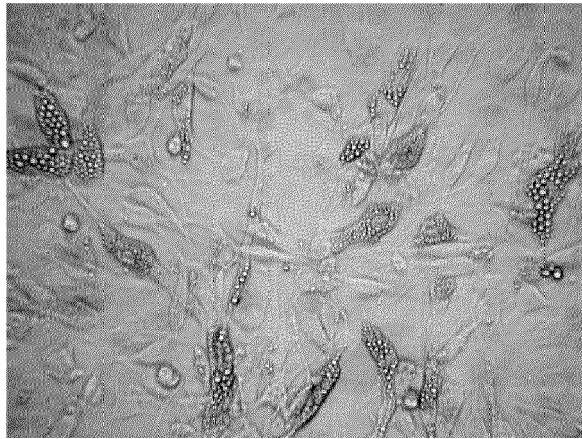
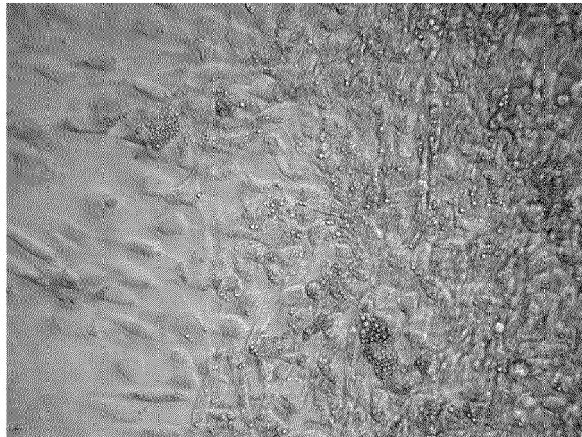


FIG. 23H



REFERENCES CITED IN THE DESCRIPTION

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